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STUDIES IN THE PHYSIOLOGY OF PARASITISM WITH SPECIAL REFERENCE TO THE SECRETION OF PECTINASE BY RHIZOPUS TRITICI

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INTRODUCTION

As a result of investigations of the relationship existing between a parasite and its host some knowledge has been obtained regarding the manner in which the infection takes place, as well as the way in which the host responds to the attack of the parasite. That some hosts are resistant to the attack of certain fungi or bacteria and not to others is well known, but why they are resistant is a subject on which there is altogether too little information. Furthermore the number of different hosts which may be parasitized by some of the so-called specialized parasites is at most only a few. The mode of infection is fairly well understood, but why a particular fungus is restricted to one or a few different hosts is not clear. On passing to parasites of a lower order the number of hosts which a particular fungus may attack is, in some cases at least, much larger. It is a well-known fact that some fungi can produce disintegration of the host tissues only after a wound has been made through which they can enter, while others have the power to penetrate the epidermis either mechanically or by the dissolution of the epidermal cell walls by enzymic action. There are, therefore, all degrees of resistance and susceptibility which apparently are dependent upon certain properties peculiar to either the host or the fungus. In other words, if a fungus can overcome the resistance of one host and not that of another it must be that the one possesses properties which the other does not. However, the experimental data at hand are altogether too limited to justify any sweeping generalizations as to what constitutes parasitism.

Rhizopus tritici Saito belongs to a large physiological group of fungi which are characterized by their ability to destroy the host quickly and to "act in advance" of their growth. It is assumed, and the evidence of the writers and of others seems to justify the assumption, that this "action in advance" is due to an enzym secreted by the fungus which

has the power to dissolve certain constituents of the cell walls so that coherence is lost.

The fungi belonging to this group kill rapidly and by destroying the middle lamellae transform the tissues into a mass of more or less isolated cells together with a large amount of a clear, somewhat colored liquid inclosed by the skin. The fungus secretes a substance which diffuses out of the hyphae into the tissue of the host and kills often several cells in advance of the fungous threads. The mycelium then must derive its nourishment from the dead material in which it is embedded. No doubt these fungi, since they live on the dead material thus produced, would be classed by some as saprophytes; nevertheless, they differ from true saprophytes in that they can attack and destroy living organisms. In this sense they are parasites.

The dissolution of the middle lamella has been demonstrated by several investigators to be due to a substance which is enzymic in nature. This enzym has been considered by most investigators to possess two rather distinct types of action on the cells of the host: first, a killing action on their protoplasmic contents, and, second, a dissolution of certain of the constituents of their cell walls. The writers at present will deal only with the latter.

The enzym which acts upon the pectic compounds of the cell walls, causing loss of coherence, has been designated by some investigators as pectinase and by others as pectosinase (*17*).[1] If it had been definitely proved that the middle lamella was largely composed of pectase the enzym which acted upon it should properly be designated as pectosinase. However, since the composition of the middle lamella has not been definitely determined and since the term pectinase is so widely used in the literature, the writers have employed it to designate the enzym secreted by *Rhizopus tritici* which dissolves the middle lamellae and produces what is termed maceration.

HISTORICAL

There is a considerable amount of data published which bear directly or indirectly on the physiology of parasitism, but only those which are germane to this phase of the subject will be considered here. DeBary (*1*) was probably among the first to detect the activity of a fungous extract in dissolving the cell wall. He showed that the drops which exuded from the sclerotia of *Sclerotinia libertiana* Fuckel., as well as the juice expressed from decayed plant tissues, contained a substance of the nature of a ferment which was thermolabile and possessed the power of dissolving certain constituents of the cell walls. He observed differences in the activity of the boiled and unboiled extracts, but he was not able to determine whether or not the entire action of the extract was due to one or

[1] Reference is made by number (italic) to "Literature cited," p. 624–625.

more substances. Similar results were obtained by Ward (*16*) and Kissling (*11*).

The work of Ward calls for special consideration. He grew Botrytis in culture and used the liquid on which the fungus grew, as well as a watery extract of the mycelium, to macerate living material and came to the conclusion that the entrance of the hyphae into the tissue depends upon the excretion of a ferment which dissolves the cellulose. Maceration was in most cases slow, which was probably due to the fact that cultures 3 weeks or more old were used. Further observations of Ward led him to conclude that the ferment sometimes accumulates to such an extent at the tips of the hyphae that the cellulose of the latter is dissolved and the ferment and protoplasmic contents of the cells pass out in the form of small droplets.

Nordhausen (*12*) used chiefly the extract from old mycelium, and came to the conclusion that the action on plant tissue was both enzymic and toxic in nature. He did not exclude the possibility that oxalic acid which was secreted by Botrytis might play a part. Behrens (*2*), on the other hand, used the expressed juice from fruits that had been decayed by certain fungi. In one case he employed the expressed juice of a pear infected with *Mucor stolonifer* Ehrb. and in another that of an apple infected with *Penicillium luteum* Zukal. He found these juices to be toxic to plant tissues even after boiling, and from these results he concluded that the toxic substance was neither volatile nor enzymic. In one case the fungus was allowed to grow three months before the extract was obtained.

Smith (*14*) compared the mycelial extract of Botrytis with weak solutions of oxalic acid on stems of lettuce. He found that the oxalic acid alone induced a macerating effect on the tissue similar to that of the extract of the fungus material, and on the basis of these results came to the conclusion that the dissolution of the cell walls noted is due to the oxalic acid secreted by the fungus.

So far as bacteria are concerned Potter (*13*) and Van Hall (*8*) found that *Pseudomonas destructans* Potter and *Bacillus omnivorus* V. Hall, respectively, produced enzyms which were able to dissolve the constituents of the cell walls of plant tissues so that coherence was lost. Potter worked both with the expressed juice of a turnip rotted with the organism and with the solution on which the bacterium had grown. He claims that a toxic substance was produced, since the boiled expressed juice from a rotted turnip caused the death of the cells of a raw turnip. He found that the organism when grown in Pasteur's solution produced oxalic acid which acts as a powerful toxic agent. It is claimed that the oxalic acid then acts as a toxin in killing the cells and that it may also play some part in the dissolution of the middle lamella. In this direction the most important work with bacteria is that of Jones (*10*), who carried on extensive researches with *Bacillus carotovorus* Jones. He found that this organism

secreted an enzym—pectinase—which has no action upon lignified or cuticularized walls, but which has the power to dissolve the middle lamella considerably in advance of the invasion of the bacteria.

Spauding (*15*) observed that in the last stages of decay produced by *Lenzites sepiaria* Fr. the middle lamellae have disappeared. Zeller (*19*) found that the same wood-destroying fungus produced a substance called pectinase capable of dissolving the middle lamella of carrots and potato disks, coherence of the tissue being entirely lost after 42 hours in an extract of the fungus powder.

Probably the most important contributions to the physiology of parasitism, at least as regards the intimate relations of the host to the parasite concerned, are those of Brown (*4*, *5*), Dey (*6*), and Blackman and Welsford (*3*).

It was shown that *Botrytis cinerea* Pers. acts in advance of its growth and that the active principle is of the nature of an enzym which dissolves certain constituents of the cell walls. It was further shown that the death of the protoplasmic contents of the cells was subsequent to the maceration of the plant tissues. To what the death of the cell is due appears to be a controverted point. Brown has been unable so far to identify two distinct principles in extracts of Botrytis mycelium. On the other hand, De Bary's (*1*), Nordhausen's (*12*), and Behrens' (*2*) conclusions seem to argue in favor of the presence of both an enzymic and toxic principle. Brown (*4*) showed that there was no dissolution of the underlying host tissues when extracts of the mycelium were placed on the unwounded surface of the host, and Blackman and Welsford (*3*) showed that the penetration through the unbroken surface of the host was accomplished by mechanical pressure. Similar results were obtained by Dey (*6*) with *Colletotrichum lindemuthianum* (Sasc. and Magn.) Bri. and Cav.

A résumé of the literature shows that various investigators have drawn quite different comclusions from the results of their experiments. Perhaps criticism of some of the earlier investigations might be justified on the grounds that they failed to describe their methods in sufficient detail to enable the reader to evaluate their results correctly. Furthermore, some of the conclusions have been drawn from the results obtained by the use of extracts of mycelium grown for a long time in culture, in some cases three weeks or more. It is probable that the material used had lost much of its power to dissolve the middle lamella, since Brown (*4*) showed that the macerating principle was most active in young and vigorously growing hyphae of *Botrytis cinerea* 1 to 2 days old. The writers also have found that the intracellular pectinase of Rhizopus is greatest in young mycelium. Harter (*9*), Dox (*7*), Young (*18*), and others showed that diastase, inulase, and other enzyms were more abundant in the hyphae just preceding the fruiting stage. It would, therefore, seem that young cultures should be employed if the maximum efficiency is to be obtained from the use of the hyphae.

METHODS OF EXPERIMENTATION

The investigations as outlined involved a study of the macerating action (*1*) of the enzym contained in the hyphae and (*2*) of that exuded from the mycelium into the culture solution. Brown (*4*) worked principally with the extract of the fungous material, although he domonstrated the excretion of the active principle into the culture medium. While offering no criticism upon the employment of the fungous extract, the writers have carried out macerating experiments with the solution on which the fungus grew as well as with the powdered hyphae. The use of the solution offered no mechanical obstacles, since to obtain fungus material the organism was grown on a liquid medium. Furthermore, in view of the fact that the macerating action is due to an enzym which diffuses out of the hyphae, it seemed to the writers that the macerating power of the solution should be studied concomitantly with that of the hyphae.

CULTURE MEDIUM

Since both the intracellular and extracellular pectinases were to be studied, a liquid medium which would support a rapid and luxuriant growth in a relatively short time and which would be available at all times of the year so that the investigations could be carried out without interruption was found to be preferable. The enzyms which diffuse out from the hyphae become dispersed throughout the medium, and after the liquor is freed of the mycelium it is ready for the macerating experiments. The medium which was finally employed was a decoction made from sweet potatoes prepared by the following formula: To the peeled potatoes add double the weight of water, steam for one hour, then squeeze out the liquid through gauze; steam a second time, filter by suction through absorbent cotton, add the required amount to flasks, plug with cotton, and autoclave for 20 minutes at 15 pounds pressure. The resulting solution is practically free of cellular structures but does contain some starch and some sugars.

In this medium a felt or mat composed of fungous threads, floating for the most part on the surface, which can readily be lifted from the liquid, is formed in from one to two days.

In view of the fact that a large quantity of mycelium was frequently necessary, 2-liter Erlenmeyer flasks containing 250 cc. of the decoction were used. If more than one flask was used the hyphae from all of them were made into one compound sample. The maceration of the raw sweet-potato disks was carried out with a weighed amount of this sample suspended in a definite amount of distilled water. Accordingly the solutions from all the flasks on which the fungus grew were collected into one flask, thoroughly mixed, and the maceration was carried out with a measured quantity.

The flasks were inoculated with spores and bits of hyphae from stock cultures which were kept in a vigorous state of growth. Incubation was carried out in the dark at a constant temperature, the temperature used depending on the character of the experiment.

UTILIZATION OF THE MYCELIUM AND SOLUTION

At the end of the incubation period the fungus growth was removed, washed in running water for 15 minutes, the water squeezed out by hand, and then treated with acetone and ether, according to Dox's (7) modification of Albert and Buchner's method for preparing "Aceton-dauerhefe." After washing, the operation is essentially as follows: The mycelium is immersed for 10 minutes in a large excess of acetone and constantly stirred, the hyphae being torn apart by forceps at the same time. The material is then squeezed to dryness and immersed in a fresh supply of acetone for 2 minutes with constant stirring. It is again squeezed to dryness and stirred for 3 minutes in ether, after which it is laid out to dry. After the odor of the ether can no longer be detected, the mycelial mass is put in flasks and stored at 9° C. until required for use.

Except in so far as it was necessary to meet the requirements of special experiments maceration of raw sweet-potato disks was carried out by the use of 0.25 gm. of the mycelium ground to a powder in fine quartz sand. The sand used for grinding was thoroughly washed in distilled water and then burned in a crucible for two hours. The enzym powder was then transferred to 120-cc. Erlenmeyer flasks, and 25 cc. of distilled water were added. Several, usually five, raw sweet-potato disks 1 mm. thick and 15 mm. in diameter were placed in each flask containing the suspension of hyphae and sand. The disks were always cut from that part lying within the fibrovascular ring of the potato, since the tissue there is more uniform, being made up largely of thin-walled parenchyma cells with only here and there a vascular bundle.

The tissue inside the fibrovascular ring of different sweet potatoes has been observed to differ somewhat in character and to react differently to the macerating agent. In view of this fact each experiment was always conducted with disks taken from the same root. The potatoes were carefully selected for soundness of the internal tissue, and any showing cavities or pithiness were rejected. In a few preliminary experiments the air was removed from the disks by suction before they were put in the solution. However, this practice was found to be unnecessary and was finally abandoned after the results showed that the rate of maceration was neither increased nor decreased by the air in the tissue.

The flasks after the disks had been added were incubated at a constant temperature, and notes were taken of the progress of maceration from time to time. Loss of coherence of the tissue was regarded as complete when the disks offered no resistance to pull. In a determination of the

end point the personal factor naturally enters, but with experience it may be determined within relatively narrow limits.

Immediately upon the removal of the fungous felt the solution was filtered through absorbent cotton to remove the remaining threads of hyphae. Cotton was used in preference to filter paper for reasons to be pointed out later. Except for special experiments to be discussed later the cultures were grown for only three days so that usually no, or only very few, spores were produced. Raw sweet-potato disks of the type mentioned above were immediately suspended in the filtered solution, incubated at the desired temperature, and records made of the progress of maceration. By this method of manipulation it is not unlikely that some spores, if produced, and some foreign organisms found their way into the solution. Pure cultures of the organisms were grown under sterile conditions, but as soon as the fungous felt was removed it as well as the solution doubtless became contaminated by foreign organisms from the air. The extracts and the solutions were so powerful in pectinase that maceration of the tissues was complete before these organisms could influence the results appreciably. In this connection it may be stated that in no case have contaminating bacteria been found to exercise any macerating action on raw disks of sweet potatoes. In cases where the macerating action was prolonged beyond two or three hours the surface of the liquid was covered with toluol. In a number of trial experiments it was found that no foreign organisms could be isolated from solutions which had been well shaken with toluol.

Other investigators in this field have carried out most of their experiments by the use of an extract of the fungous material. This is usually obtained by grinding the mass of hyphae, suspending it in water for a definite length of time, then filtering out the fungous débris. This method was not followed by the writers for reasons which will be made clear later. The ground mycelium was suspended in the water and formed a part of the system to which the disks were added. Several objections may be offered against the extraction of the hyphae. First, it allows foreign organisms, which doubtless are introduced during the process of manipulation, to multiply. Second, a considerably longer time is required to complete an experiment and, third, all the enzym may not be extracted and some of it may be removed by the filter paper.

EXPERIMENTAL DATA

PECTINASE FORMATION BY RHIZOPUS TRITICI

At the outset of the storage-rot experiments it was sometimes difficult to obtain a culture of Rhizopus from rotted sweet potato, although there was an abundance of evidence that this fungus caused the decay. In order to avoid foreign organisms the plate plantings were made from partially decayed tissue adjacent to the healthy zone. Plates so made

were frequently sterile, which at once suggested the possibility that there was disintegration of the tissue or "action in advance" of the growth of the hyphae. Cultures made a little more remote from the healthy tissue usually gave a pure culture of Rhizopus. Following up the clue suggested by the plate plantings, preliminary macerating experiments were made with the solution on which the fungus grew and with extracts of the hyphae, both of which resulted in the complete disintegration of raw sweet-potato disks within a few hours.

Literally hundreds of macerating experiments have been carried out with the enzym contained in the hyphae and with that exuded into the substratum. Because of the large number of experiments detailed elsewhere dealing with special phases of the subject and which contribute additional evidence of the occurrence of pectinase in the mycelium and in the solution on which it grew, only a résumé of the process of maceration will be described here. The raw sweet-potato disks, usually five, of uniform size (1 mm. thick by 1.5 cm. in diameter), were dropped into the solution or into the hyphal suspension, as the case might be, both of which were prepared according to methods already described. The incubation was usually carried out at 37.5° C., although higher or lower temperatures, depending upon the object of the experiment, were sometimes used. In some of the more powerful preparations a change in the appearance of the disks frequently took place in from 10 to 15 minutes after they were added to the solution. The disks became somewhat cupped or twisted in shape and slightly crisp, brownish in color, probably due to oxidation. Approximately ½ hour thereafter, depending somewhat upon the macerating power of the enzym, the disks became flaccid. This was followed by softening of the tissue at the surfaces and edge. The progress of the softening continued gradually until the disks were softened throughout so that they offered no resistance to pull. A microscopic examination of completely macerated tissue shows that the cells are separated along the line of the middle lamellae but that the cells themselves remain intact.

The time required to macerate the disks completely varied from about 1 to 5 hours. Several factors were found to influence the rate of maceration. No two preparations could be regarded as exactly alike, although the cultures were grown and the solutions and hyphae treated thereafter under as nearly identical conditions as possible. Again it was found that the end point of maceration varied with the different disks in the same system, although they were all cut from the same sweet potato and from the same portion of the potato. It was found, therefore, that something must be left to the judgment of the observer. However, after considerable practice in the determination of the end point it is possible to determine it within relatively narrow limits. These controlled and carefully conducted experiments show that *Rhizopus tritici* produces a powerful intracellular and extracellular pectinase which can dissolve

the middle lamellae of sweet-potato disks so that the cells fall apart without the cells themselves undergoing any apparent mechanical alteration.

INFLUENCE OF THE AGE OF THE MYCELIUM ON THE RATE OF MACERATION

Brown (*4, I*) showed that the young, growing hyphae of *Botrytis cinerea* contained a more powerful enzym than old hyphae, and he used for most of his work an extract of the fungus grown for only 24 hours. Other investigators have obtained similar results for other enzyms of various fungi. It appears to be generally agreed that the most powerful extract is obtainable from young or comparatively young hyphae, but it can not be expected that all fungi will show the same limitations as regards the length of time for growth. This being a question of considerable importance with respect to much of the future investigations in this direction, experiments were planned and carried out in considerable detail for the purpose of determining at what period in the growth of the organism the most powerful extract could be obtained from the hyphae, and likewise when the maximum amount of the enzym was present in the solution. While it may be argued that comparative studies do not necessarily require that the work be done with hyphae and solutions containing the maximum amount of enzym, there are several reasons why it is desirable to do so. First, the time required to complete maceration is shortened so that the experiments may be completed within a single day. Second, a short period of maceration eliminates to a large extent the influence that might be exercised by the introduction of contaminating organisms. Third, infection is essentially dependent upon the extracellular enzym of young hyphae, at least in so far as enzyms play a part.

To determine the period of maximum content of enzym in the hyphae and in the solutions, 50 flasks containing sweet-potato decoction and 36 inoculated with spores of *Rhizopus tritici* were prepared. All the flasks were incubated at 37.5° C. in the dark. At the end of each 24-hour period, with exceptions to be noted in the following table, 4 of the flasks (3 inoculated and 1 not inoculated) were taken from the incubator, the fungous felt was removed, and the solution was filtered through absorbent cotton. The hyphae from the inoculated flasks were treated in the usual way with acetone and ether, and after drying the fungous material was made into one compound sample. Duplicate 0.25-gm. samples of the hyphae were weighed out, ground in sand, suspended in 25 cc. of distilled water, and used for the maceration of the sweet-potato disks. A water blank containing no enzym powder was used as a control. The incubation temperature was 37.5°. Records were made from time to time of the progress of maceration.

The contents of one of the three flasks on which the fungus had grown were steamed to deactivate the enzym, and together with one flask not

inoculated they served as a control. Maceration was carried out at 37.5 C. The results of these experiments are shown by Table I.

TABLE I.—*Time in hours required by the mycelium and the solution on which it grew to macerate sweet-potato disks after growth in sweet-potato decoction for stated intervals of time*

	Hours required to macerate after growing in culture—											
	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	10 days.	11 days.	12 days.	13 days.	16 days.
Powdered hyphae:												
Enzym powder	3.0	4.00+	4.5	4.5	4.75	5.5 +	5.5 +	5.5+	5.25	7.0 +	8.5	19.0
Water (control), no maceration.												
Solution:												
Not steamed	3.0	1.75	1.75	1.75	1.75	1.75	1.75	2.0+	1.5	2.25+	4.5	6.0+
Steamed (control), no maceration.												
No fungous growth (control), no maceration.												

From Table I it is seen that the macerating power of the hyphae per unit of dry weight is greatest on the first and second days of growth and becomes gradually weaker thereafter. The enzym of the solution, on the other hand, is equally powerful from the second to the seventh day of growth, inclusive. The writers have arbitrarily adopted three days as the standard length of time for growth. Somewhat discordant results were obtained in the steamed solutions and in the solutions on which the fungus was not grown. No maceration was apparent in such solutions at the end of the time required to macerate completely the disks in the solutions containing the active enzym. On the other hand, in some cases if they were exposed for some hours longer maceration was evident, and it was often completed in 24 hours.

RELATION OF VARIOUS FACTORS TO MACERATION

The writers have previously discussed the method used in carrying out macerating experiments with the enzym contained in the mycelium and with that exuded into the substratum. Experimental data have also been submitted which show that *Rhizopus tritici* produces both a powerful intracellular and extracellular pectinase which dissolves the middle lamellae of raw sweet-potato disks in from two to five or more hours (Table I), depending upon the length of time the organisms grew in the culture. It was shown that the most vigorous enzym was contained in cultures 1 to 3 days old.

In investigations of this kind the methods used in the treatment of the fungous material or of the solution might conceivably greatly influence the activity of the enzym. To obtain reliable data it was, therefore, necessary to make a careful study of the physical, mechanical, and other factors involved in the various steps in the process of manipula-

tion to determine whether they altered the macerating power of the enzym, and if so, to what degree.

WASHING THE MYCELIUM.—The first step in the treatment of the fungous growth as finally adopted consisted in washing the felt in running tap water for about 15 minutes, squeezing out the water, and subsequently treating it with acetone and ether. Washing in water for this length of time did not remove any of the macerating principle from the mycelium, as is shown by the following experiments. Several fungous mats (two days' growth) about 6 inches in diameter were removed from the culture flasks and rinsed for 2 or 3 seconds in running water to remove some of the adhering culture fluid. The felts were then cut into two parts, one of which was washed for 15 minutes in flowing water. Both lots were subsequently treated with acetone and ether in the usual way. A comparison of the macerating power of the two lot of material was made by using 0.25 gm. of the fungous hyphae ground in sand and suspended in 25 cc. of distilled water. Several sweet-potato disks (1 by 15 mm.) added to the systems and incubated at the same temperature were macerated in an equal length of time.

ACETONE-ETHER TREATMENT.—The treatment of the mycelium with acetone and ether did not influence the macerating power of the hyphae, as was shown by a comparison of the corresponding halves of the same felts, one of which was dried over calcium chlorid in partial vacuum for five hours and the other treated in the usual way with acetone and ether. After the felts of each set were thoroughly dried they were mixed into a compound sample, and 0.5 gm. of the powdered mycelium of each was suspended in 25 cc. of distilled water. Sweet-potato disks (1 by 15 mm.) were macerated in equal time in an enzym powder of the same concentration for the two samples. From these results it would seem that the treatment of the mycelium with acetone and ether in no way influences the macerating power of the hyphae.

Probably the chief virtue in the acetone-ether method lies in the ease with which the dried mycelium can be handled subsequently. Rhizopus forms a very compact, leathery mat which is difficult to handle if allowed to dry before the threads are torn apart. The practice has been to pull the hyphal threads apart while the material is submerged in the acetone and ether, so that by the time the treatment is finished the threads are well separated and broken into short lengths. When dried the material is loose and cottony in general appearance, so that a quick and accurate weighing is possible.

GRINDING THE HYPHAE BY SAND.—A comparison of the rate of maceration by mycelium ground in sand with mycelium not so ground showed that, everything else being identical, the disks in the suspension of ground hyphae reached complete maceration a little ahead of those in the solutions with unground hyphae.

The quantity of sand used for grinding the mycelium does not influence the rate of maceration, as is shown by the results of comparative experiments in which several uniform lots of mycelium were ground with 0.25, 0.5, 1.0, 5.0, and 10.0 gm. of sand, respectively, and transferred to small Erlenmeyer flasks. To each flask were added 25 cc. of distilled water and immediately the usual sized sweet-potato disks. Maceration carried out at 37.5° C. was complete in all samples ground in sand in 3.5 hours. In the unground samples which served as controls maceration was complete in 4 hours.

TOLUOL AS AN ANTISEPTIC.—In experiments where a considerable length of time was required to complete maceration, about 2.5 cc. of toluol, or enough to cover the surface of the liquid, was used as an antiseptic. Usually antiseptics were not employed, since maceration was completed before foreign organisms became sufficiently abundant to influence the results materially. Sweet-potato disks immersed in pure toluol for 19 hours showed no evidence of maceration. The use of toluol as an antiseptic in flasks containing enzym powder did not retard the maceration of sweet-potato disks when compared with flasks to which no toluol was added, maceration being completed in each case in an equal length of time.

EXTRACTION OF THE HYPHAE.—The usual method in investigations of this kind is to extract the powdered mycelium with water for a given time, filter off the solid matter, and subject the disks or material to be macerated to the extract. Two objections may be offered to this method. First, it requires a considerably longer time to carry out the experiments and, second, all of the enzym may not be removed from the hyphae. A considerable number of experiments which need not be related in all their details were conducted to determine whether extraction of the hyphae was necessary or desirable. A comparative study was made of the rate of maceration of 0.25 gm. of hyphae extracted for 18 hours in 25 cc. of water at 9° and at 37.5° C., respectively, and of hyphae not previously extracted. The results of these experiments showed that the disks added to the unfiltered solutions and incubated at 37.5° in both the extracted and nonextracted mycelium were macerated in the same length of time.

FILTERING.—Since extraction of the mycelium was found unnecessary, filtering the solutions free of the mycelial particles and sand was not practiced. Suffice it to say in this connection that the several experiments conducted showed that filtering the solution through one and two thicknesses of No. 2 Whatman chemically prepared filter paper considerably weakened the extract. To just what the weakening of the enzym was due was not determined, but it is probable that some of the enzym was retained in and adhering to the fungous particles and sand which were caught by the filter paper.

On the other hand, filtering the solution on which the fungus grew gave different results. Although most of the fungous material could be lifted from the flask, filtering was necessary before one could be reasonably sure that no hyphae remained in the solution. Aside from the hyphae which were left in the solution, the liquid was reasonably clear and free of solid particles. To determine whether filtering removed any of the macerating principle, a number of experiments were made with solutions on which the organism had grown for about 40 hours at 37.5° C. The contents of two large flasks from which the fungous growth had been removed were mixed into one compound sample, one portion of which was filtered through a No. 2 Whatman chemically prepared filter paper, and another portion through a thick layer of absorbent cotton. The remainder served as a control, a portion of which was steamed 15 minutes to inactivate the enzym. Raw sweet-potato disks were then added to the different solutions and incubated at 37.5°. The results showed that the disks were macerated as quickly in the solution filtered through filter paper and cotton as in the unfiltered solution.

CENTRIFUGING.—Some investigators have removed the sand and fungus débris from the solution by centrifuging, a method well adapted to this type of work if an extract is required. This method is possibly open to the same objection as filtering the extract through filter paper in which it was shown that some of the macerating principle was removed. A comparison was made of the macerating power of the supernatant liquid of a centrifuged solution with that of the solutions of the same origin filtered through filter paper, through cotton, and not filtered. The experiment was carried out by the use of 0.5-gm. samples of hyphae ground in sand and extracted for 18 hours in 25 cc. of distilled water. At the end of 18 hours the contents of some of the flasks were centrifuged for 5 minutes, and the supernatant liquid was used for macerating experiments. The contents of some of the other flasks were filtered either through filter paper or through cotton. The remaining flasks were not filtered and served as controls. The results showed that maceration was complete in the solution not filtered or centrifuged in 3.5 hours, in the centrifuged solution in 4 hours, and in the filtered solutions in 5 hours.

RELATION TO SUNLIGHT.—Light is said to be injurious to certain enzyms. In view of the fact that most of the mechanical operations of the present investigations must be carried out in diffused light, in some cases the enzym powder being exposed to the light for a considerable length of time, it was thought advisable to determine what effect, if any, such exposure had on the macerating power of the enzym. These experiments were carried out by exposing for 2 hours lots of 0.25 gm. of hyphae to direct sunlight and diffused light and placing some in an incubator in which light was practically excluded. In some experiments

the mycelium was ground before it was exposed to the light. At the end of the period of exposure the powdered hyphae were placed in an incubator for 18 hours at 9° C. The mycelial powder was then suspended in 25 cc. of distilled water, and after sweet-potato disks of the usual type had been added, incubation was carried out at 37.5°. Records taken from time to time of the progress of maceration showed that the different light intensities used had no influence upon the macerating principle. Grinding before exposure to light was likewise without effect, maceration being completed in 4.5 hours in all cases.

QUANTITY OF SOLUTION.—It is desirable, other things being equal, to employ technic in investigations of this kind which will require the least possible time in manipulation. If the volume of the solution whose macerating property is to be studied should influence the rate of maceration, a measured quantity would be required for all comparative work. This would require time in measuring. Without going into the details of the results of this phase of the work it may be stated that there was no difference in the time required to macerate sweet-potato disks in a solution on which the fungus grew in volumes varying from 25 to 100 cc.

CONCENTRATION OF SOLUTION.—As would be expected, diametrically opposite results were obtained as regards the concentration of the solution, the rate of maceration being most rapid in the solutions containing the largest quantity of enzym powder.

OPTIMUM TEMPERATURE FOR MACERATION.—It is a well-known fact that temperatures below the optimum progressively retard the action of enzyms. Above the optimum, action is retarded more rapidly. With respect to the macerating principle found in *Botrytis cinerea*, Brown (5) showed that at 65° C. deactivation was practically instantaneous.

The investigations of the writers with respect to this subject were made with a solution obtained from six 2-liter flasks on which the fungus had grown for 48 hours at 37.5° C. The solutions were filtered through two thicknesses of cheesecloth and combined into one large receptacle and thoroughly mixed. Twenty-five cubic centimeters of this solution were pipetted into small flasks and exposed for 1 hour at 9°, 20°, 30°, 40°, 45°, 50°, 55°, and 60°, respectively, to bring to constant temperature. Raw sweet-potato disks were then added to the flasks, and notes were taken from time to time of the degree of maceration. At 9° maceration was completed in 3.5 hours, and the time was progressively shortened with an increase of the temperature up to the macerating optimum. At 60° the enzym was completely deactivated and no maceration took place. The optimum temperature has been difficult to establish, but it lies somewhere between 45° and 55°, and probably nearer 45° than 55°. If more refined methods were at hand for determining the end point the optimum might probably be more nearly established. Above 55°

deactivation of the enzym was very rapid, and below 45° its action was retarded. Experiments with the ground hyphae in which 0.25-gm. lots were suspended in 25 cc. of distilled water and exposed to the same temperatures as the solutions discussed above gave similar results. In recapitulation it may be said that the intracellular and extracellular pectinase bear the same relation with respect to the optimum temperatures for maceration.

SUMMARY

(1) *Rhizopus tritici* produces a powerful intracellular and extracellular pectinase when grown in sweet-potato decoction.

(2) The enzym is able to effect the complete maceration of raw sweet-potato disks so that coherence of the cells is entirely lost.

(3) The optimum temperature for maceration is between 45° and 55° C. At 60° deactivation of the enzym is nearly instantaneous; below 45° the activity of the enzym decreases simultaneously with the decrease in temperature.

(4) The maximum enzym content of the hyphae and the solution is attained in about 24- and 48-hour-old cultures, respectively.

(5) The volume of the enzym solution of a given strength does not influence the rate of maceration; the concentration of the enzym in the solution does.

(6) Exposure of the hyphae for 2 hours to direct sunlight does not affect its macerating power.

(7) Centrifuging to remove the sand and fungous débris slightly deactivates the enzym.

(8) Filtering the solution in which the powdered hyphae and sand are suspended through filter paper weakens the enzym; filtering the solution after the removal of the fungous felt does not reduce its strength.

(9) Extraction of the powdered hyphae for 18 hours in water does not increase the rate of maceration when compared with hyphae not extracted.

(10) Toluol may safely be employed as an antiseptic without impairing the action of the enzym.

(11) The quantity of sand used for grinding the hyphae does not influence the action of the enzym.

(12) The treatment of the hyphae with acetone for 12 minutes and ether for 3 minutes has no influence on the macerating action of the hyphae.

(13) Washing the hyphae in running water for 15 minutes has no influence on the action of the enzym.

(14) The results of these investigations indicate that work of this type involving a study of the relationship existing between a host and its parasite may throw some light on the important question of parasitism.

Rhizopus tritici belongs to a large group of organisms, incapable of themselves of penetrating the unbroken cells of the epidermis mechanically or of dissolving them with its enzyms. However, after it has once reached the tissues beneath the epidermis, it progresses with great rapidity. It, like certain other organisms, is characterized by its ability to "act in advance" of its growth.

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RESPIRATION AND CARBOHYDRATE CHANGES PRODUCED IN SWEET POTATOES BY RHIZOPUS TRITICI

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INTRODUCTION

These investigations had for their purpose to determine (1) the relative rate of respiration, as measured by the carbon dioxid (CO_2) given off by the two halves of sweet potatoes, one of which was rotted by *Rhizopus tritici* Saito, and (2) how the starch, cane sugar, and reducing sugar content of the two corresponding halves differed at the end of the experiment. It was believed that an insight into the physiological changes of the host brought about by the fungus might be obtained by experiments in which the carbohydrate content and respiration were determined simultaneously.

METHOD OF EXPERIMENTATION

RESPIRATION

The measure of the CO_2 output was determined from the corresponding halves of the same root by four separate experiments. Large, sound sweet potatoes were carefully washed, cut in two longitudinally, and pared until the two parts had the same weight. A small "well" was made in each half by means of a cork borer about 1 cm. in diameter. One half was inoculated by pouring a 24-hour growth of the organism in sweet-potato decoction (about 2 cc.) into the "well," and an equal volume of sterile water into the "well" of the control half. The two halves were then put into separate air-tight desiccators which served as respiration chambers through which CO_2-free air was drawn. The CO_2 was collected in trap bottles containing barium hydroxid ($Ba(OH)_2$) and determined as barium carbonate ($BaCO_3$). The method used for the removal of the CO_2 from the air and for its collection and subsequent determination is essentially the same as that used by the writers (*5*) [1] in the study of the respiration of fungi in nutrient solutions, to which the reader is referred for details. The respiration chambers were kept in an incubator maintained at a constant temperature of 30° C. The CO_2 given off was determined every 24 hours. By this method decay of the inoculated half was evident in 24 to 48 hours and usually complete in about 3 days. As soon as decay was complete the two halves were removed from the respiration chambers, weighed, and prepared for analysis.

[1] Reference is made by number (italic) to "Literature cited," p. 634–635.

ANALYSIS

The halves were prepared for analysis by first being run through a pulping machine. The pulp was then thoroughly mixed, and duplicate samples were transferred to tared weighing bottles for dry-weight determinations. The pulp was covered with 95 per cent alcohol and then dried to constant weight in a vacuum oven at 60° C. Another set of duplicate samples was taken from the pulp of each half for carbohydrate determinations. For this purpose 10-gm. portions of the pulp were placed in beakers containing about ½ gm. of calcium carbonate ($CaCO_3$) and were then covered with 70 per cent alcohol and heated to boiling. The contents of each beaker were then transferred to a tared thimble and extracted with 70 per cent alcohol in a Soxhlet's extraction apparatus for about seven hours. The extract was made up to 500 cc. by the addition of water, and 100 cc. were transferred to a beaker and evaporated to about 25 cc. It was then transferred to a 100-cc. sugar flask and cleared in the usual way with lead acetate. The cane sugar was hydrolyzed according to the method of Davis and Daish (*4*), and it and the reducing sugars and hydrolyzed starch were determined according to the method of Clark (*2*).

After the sugars were extracted the solid material was dried to constant weight in a vacuum oven at 60° C. The pulp was finely ground in a mortar, and a known amount was transferred to a 100-cc. volumetric flask and the starch hydrolyzed by boiling in a 5 per cent solution of hydrochloric acid (HCl) for 2½ hours with a reflux condenser. It was then neutralized by sodium hydroxide (NaOH), made up to volume, filtered, and the starch determined as reducing sugar.

The Yellow Jersey variety of sweet potatoes grown on the Potomac Flats near Arlington, Va., was used for all these investigations.

All the data were calculated to the original wet weight of the material used. The directly reducing sugar was calculated as glucose.

It might be urged that the results both of the respiration experiments and of the carbohydrate determinations were vitiated by the presence of microorganisms other than *Rhizopus tritici*. It is not unlikely that bacteria were present on the surface and that they would contribute something to the total of the CO_2 given off. Before the samples were put into the respiration chamber they were thoroughly washed but were not disinfected. The length of the respiration period was three days in all experiments except one, which continued another day longer. Cultures were made from the rotted potato at the close of the experiments, and a pure culture of *R. tritici* was obtained in every case. The writers do not believe that bacteria or fungi were present in sufficient amount to alter materially either the results of the respiration or the carbohydrate content. As a matter of fact, all respiration experiments of a sort where sterile conditions can not be employed are subject to alterations due to the presence of foreign organisms.

EXPERIMENTAL DATA

RESPIRATION

The results of all the respiration experiments expressed in grams of CO_2 given off for each 24-hour period as well as the total for the duration of the experiment are given in Table I.

TABLE I.—*CO_2 output from a decaying and a sound half of the same sweet potato at the end of each 24-hour period* [a]

Experiment No.	Sample.	First day.	Second day.	Third day.	Fourth day.	Total.
605	Control	0.6210	0.4807	0.4652	.....	1.5669
	Rotted	1.4374	4.1653	4.2861	.....	9.8888
609	Control	.9144	.9147	.9145	.....	2.7436
	Rotted	1.9583	8.5934	8.7363	.....	19.2880
631	Control	.7153	.4986	.4807	.....	1.6946
	Rotted	1.0726	5.0479	6.5600	.....	12.6805
637	Control	.3910	.4173	.3502	0.4287	1.5872
	Rotted	.9813	3.9748	4.1859	3.3626	12.5046

[a] Expressed in grams.

It will be noted that the CO_2 output at the end of the first 24 hours was considerably greater in the decaying than in the control half. This difference was further increased during the next 2 days. In the first three experiments decay was completed in 3 days and in the last one in 4 days. As shown by the last column a total of from 6.3 to 7.8 times as much CO_2 was evolved by the decaying half as by the control.

The figures presented in Table I should not be interpreted as representing exactly what happened in each individual case. In other words, an allowance should be made for variations in respiration that would normally take place between two sound halves of the same potato of equal weight. It was shown by Hasselbring and Hawkins (7) that wounding accelerates the CO_2 output of sweet potatoes. Preliminary to the experiments, the results of which are recorded in Table I, the daily evolution of CO_2 from the corresponding halves of several sound sweet potatoes was determined for the purpose of ascertaining the probable error. The two halves were held under similar conditions, and the CO_2 was collected for 24-hour periods. The greatest variation was about 11 per cent, but in most cases it was very much less. If a correction of this variation were made in each individual experiment it would not be sufficient to alter materially the ratio of the CO_2 output between the rotted and control halves.

CARBOHYDRATE CHANGES

IN LIVING POTATOES.—As soon as decay was complete the halves were removed from the respiration chambers, pulped, and prepared for the determination of starch, cane sugar, reducing sugar, and percentage of moisture. The results of these analyses are shown in Table II.

TABLE II.—*Carbohydrate content of the healthy and rotted halves at the close of the respiration period*

Experiment No.	Sample.	Moisture.	Reducing sugar as glucose.[a]	Total sugars as glucose.[a]	Cane sugar.[a]	Starch.[a]	Total loss of reducing sugar equivalent to CO_2 evolved.	Total carbohydrates as glucose not accounted for as CO_2.
		Per cent.					*Gm.*	*Gm.*
605	Control	67.40	3.9330	45.880	39.845	196.851	.....	.....
	Rotted	71.95	4.2424	10.206	5.525	165.083	6.7425	3.9699
609	Control	68.25	4.8709	55.308	47.915	168.541	.....	.....
	Rotted	73.55	8.6419	13.827	4.926	147.298	13.1509	4.4414
631	Control	71.30	6.4365	52.718	43.967	166.205	.....	.....
	Rotted	76.70	4.7507	25.440	19.655	127.260	8.6457	8.0133
637	Control	73.50	6.3600	70.800	61.220	166.135	.....	.....
	Rotted	74.60	4.9000	19.615	13.980	153.925	8.5258	5.1474

[a] Expressed in milligrams per gram of original wet weight.

The results of these analyses show that the starch, total sugars, and cane sugar are lower in the rotted than in the sound half of the potato. In the first two experiments the reducing sugars are increased and in the last two decreased. It is, of course, not possible to state from these experiments alone in what form the carbohydrates are utilized by *Rhizopus tritici*. Other investigations by the writers showed that glucose is readily utilized and cane sugar sparingly or not at all. Furthermore, in nutrient solutions this fungus will thrive better with boiled starch as a source of carbon than when cane sugar is used. On the other hand, raw starch in culture solutions (5) is hydrolyzed to some extent by *R. tritici*. Hawkins (9) found that certain species of Fusarium reduced the sucrose and reducing-sugar content of the Irish potato while the starch content was not altered and that in the peach the brownrot fungus increased the reducing sugars and the total sugars and decreased the cane sugar.

It would seem from the experiments of the writers that a fairly constant supply of reducing sugars was maintained and that the source of this supply was the cane sugar and starch.

The total amount of CO_2 evolved is shown in the last column of Table I, and in column 8 of Table II the reducing sugars equivalent to the CO_2 output is given. The total loss of the carbohydrates (starch, cane sugar, and reducing sugar) as determined from the analyses is seen to be greater than that which is accounted for by the CO_2 given off. In the last column of Table II is shown in grams the loss of carbohydrates in excess of that which can be accounted for by the CO_2 output.

What becomes of the carbohydrates can be only a matter of speculation. Hasselbring and Hawkins (7), in respiration work with sound sweet potatoes, found that the cane sugar accumulated slightly and

suggested the possibility that some of the reducing sugars may have been utilized in the manufacture of sucrose. From the results of these investigations, however, it will be necessary to search elsewhere for an explanation, since the cane sugar as well as the starch content was reduced.

In investigations of this type where microorganisms are involved, several possibilities may be advanced to account for the loss of the carbohydrates in excess of that required to satisfy the CO_2 given off. First, a portion of it undoubtedly was utilized in the construction of fungous material. No measure could be made of the fungous material formed, and although a considerable amount of mycelium was produced the actual dry weight would be small. Second, Harter and Weimer (*6*) showed that in Czapek's nutrient solution with glucose as the only available carbohydrate *Rhizopus tritici* and other fungi produced a measurable quantity of alcohol. Similar results have been obtained by other investigators with other fungi. In some cases as much as 8 per cent alcohol was produced. Probably the physiological reaction of a fungus is different when grown on a sweet potato and on an artificial medium. However, if alcohol was produced in the one case there are reasons to suspect that it might be formed also in the other. As a matter of fact, the writers have demonstrated by the use of the iodoform test the presence of alcohol in rotted sweet potatoes. Third, acid production has been demonstrated by many investigators, especially when nutrient solutions were employed. Cooley (*3*) found that *Sclerotinia cinerea* (Bon.) Schroet. formed oxalic acid when grown upon peach juice, and Hawkins (*8*) that the acid content of the rotted half of the peach, when decayed by the brownrot fungus, was higher than that in the sound half. Behrens (*1*), on the other hand, found that the acid content of the sound portion of an apple was higher than that of the rotted half. Hydrogen-ion determinations were made of the expressed juice of some of the rotted and decayed halves used in these experiments at the close of the respiration period. The results of the determinations of the samples in two experiments gave a P_H of 3.71 and 3.695 for the rotted and 5.44 and 5.032 for the sound.

That acids are produced in considerable abundance seems quite evident, and that alcohol is formed seems probable. The carbohydrates required for the manufacture of acids and alcohol, together with that utilized directly in the production of fungous material, will probably account to a large extent for the decrease in the sugars and starch which are not accounted for by the CO_2 evolved.

The foregoing results show that the cane sugar disappears at least in part from the sweet potato when decayed by *Rhizopus tritici*, indicating that it was either used directly by the fungus or converted by the host into some other compound, possibly reducing sugar. Studies of the

growth of this fungus in nutrient solutions with cane sugar as the only source of carbon have shown that this substance is used only sparingly.

This fact, together with the evidence contained in the data already submitted, indicates three possibilities: First, that the fungus can utilize to a better advantage cane sugar when other carbohydrates are present; second, that in the living potato the host under the stimulation of the fungus inverts the cane sugar; and third, that the acids produced by the fungus invert the cane sugar. The following experiments were designed to throw some light on this phase of the subject.

IN COOKED POTATOES.—Large sweet potatoes were cut longitudinally into two parts and pared to equal weight. The two halves were cut in small blocks into separate flasks and sterilized by steaming on three consecutive days. One flask was inoculated with *Rhizopus tritici* and the other one held as a control. They were both kept in an incubator held at a constant temperature of 30° C. After 15 days the starch, cane sugar, and reducing sugar of the control and inoculated flasks were determined according to the method already described. The results of these analyses are shown in Table III.

TABLE III.—*Carbohydrate content of inoculated and uninoculated cooked sweet potatoes*[a]

Experiment No.	Sample.	Reducing sugars as glucose.	Total sugars as glucose.	Cane sugar.	Starch.
670	Control	45.56	122.77	73.36	99.565
	Inoculated	0	9.02	8.569	15.525
740	Control	44.21	110.42	62.90	97.477
	Inoculated	74.025	73.685	0	14.424

[a] Calculated to milligrams per gram of original wet weight.

An inspection of Table III shows that the starch, cane sugar, and total sugar content of the inoculated material was greatly reduced, the cane sugar having disappeared entirely in experiment 740. It seems from these results that the fungus can either use cane sugar directly or convert it into some other substance before using. With respect to the reducing sugars the results of the two experiments do not parallel. In experiment 670 no reducing sugar remained in the inoculated flask, whereas in experiment 740 there was an actual increase over that of the control. This apparent discrepancy may be explained at least in part if one takes into consideration the growth of the fungus in the two cases. It was previously pointed out that glucose is an excellent source of carbon for this fungus, supporting in a nutrient solution an abundant mycelial growth. The growth of the mycelium in the first experiment was very luxuriant, about half filling a 2-liter flask, while in the second very little aerial growth took place. In view of these facts it is likely that in the former case the reducing sugars

were utilized largely in supporting an energetic respiration and in the construction of fungous material. In the second experiment with a smaller amount of fungous growth a correspondingly less amount of sugar would be utilized in supporting respiration and in the construction of fungous material. The conversion of the cane sugar and starch into reducing sugars probably took place faster than it was utilized; hence its accumulation.

Further interesting facts as regards the availability of monosaccharids and disaccharids in supporting the growth of this fungus are shown by experiments in which cane sugar and glucose alone and in combination are used as the only sources of carbon in Czapek's nutrient solution.

IN NUTRIENT SOLUTIONS.—For each experiment three flasks were prepared to contain a modification (*10*) of Czapek's nutrient solution and 1 per cent of glucose and cane sugar alone and in combination as the only source of carbon. The solutions were then inoculated, and the fungus was allowed to grow for 17 days. At the end of the growth period the mycelium was removed and the dry weight of fungous material determined. The reducing sugars and cane sugar were again determined. The loss of glucose and cane sugar as well as the dry weight of the fungous growth are shown in Table IV.

TABLE IV.—*Loss of glucose and cane sugar and amount of fungous material produced*

Experiment No.	Carbohydrates present.	Loss of dextrose.[a]	Loss of cane sugar.[a]	Dry weight of fungous material.
				Gm.
640	Glucose	52.347	0	0.3954
	Glucose and cane sugar	0	60.68	.3583
	Cane sugar	0	12.118	.2430
656	Glucose	57.99	0	.5113
	Glucose and cane sugar	0	60.09	.5158
	Cane sugar	0	0	.0853
669	Glucose	63.38	0	.5253
	Glucose and cane sugar	4.53	59.49	.5060
	Cane sugar	0	5.713	.1514

[a] Expressed in milligrams per 10 cc. of solution.

The results of these experiments show that when cane sugar is the only source of carbon a small amount of fungous material is produced. In experiment 656 there was no loss of cane sugar, and in each of the others the loss was slight. These results would seem to indicate that cane sugar when present alone in the solution is not readily utilized. On the other hand, when cane sugar and glucose are added together in the solution in equal amount, the loss of the former is relatively large but the latter remains practically constant. When, however, glucose is the only source of carbon there is a considerable reduction in the amount present at the end of the experiment.

These experiments show, then, that the cane sugar disappears when it is in combination with glucose, but that it is sparingly utilized when it is the only source of carbon. It has been shown that the growth of *Rhizopus tritici* renders the solution as well as the sweet potato more acid. In view of these facts it is probable that the increased acidity of both the decayed potato and the solutions is at least in part responsible for the inversion of the cane sugar.

SUMMARY

(1) The relative amount of CO_2 given off from the two halves of the same sweet potato, one of which was decayed with *Rhizopus tritici*, was determined. It was found that the decayed half gave off a total of from 6.3 to 7.8 times as much CO_2 as the healthy. Decay was completed in three days in all experiments except one which continued for one day longer.

(2) At the close of the experiment the starch, cane sugar, and reducing sugar were determined in the healthy and decayed halves. There was a smaller amount of starch, cane sugar, and total sugars in the decayed sample than in the healthy one, while the reducing sugars were increased in two experiments and decreased in two.

(3) The total quantity of the carbohydrates (starch, cane sugar, and reducing sugars) lost in the decayed sample, according to calculations based on the analytical data, is greater than that lost through respiration, as measured by the CO_2 given off. It seems, therefore, that a portion of the carbohydrates was utilized in other processes such as the production of fungous material, acids, alcohol, etc.

(4) When the fungus was grown on cooked potatoes (sterile) a reduction in the starch, cane sugar, and total sugar similar to that of the living potatoes took place.

(5) In Czapek's nutrient solution glucose was readily utilized when it was the only source of carbon available. When glucose and cane sugar were combined in the solution there was a reduction only in the amount of cane sugar. When cane sugar was the only available source of carbon it seemed not to be utilized by the fungus to any extent.

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WOUND-CORK FORMATION IN THE SWEET POTATO

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INTRODUCTION

The investigations reported in this paper were designed primarily to determine to what extent and under what conditions wound-cork is formed by the sweet potato and whether or not its formation serves in any way as a barrier to infection by *Rhizopus tritici* Saito.

PERIDERM FORMATION

The sections made from samples taken at the beginning of the experiments showed the condition of the tissue before any reaction to the wound stimulus occurred. The sections are made up of thin-walled cells varying somewhat in size but in the main rather large, with here and there a vascular bundle. The cells, with the exception of those comprising the vascular tissue, are more or less uniformly filled with starch. After 24 hours' exposure in a moist chamber held at room temperature (about 25° C.) a distinct change is already noticeable. A layer of cells running parallel to the exposed surface, varying from 3 to 10 deep, contains more starch than do those immediately beneath. No other change can be detected at this time, but on the second day the starch in the lower layer of cells becomes conspicuously less. About this time one or two indistinct cross walls are laid down in some of the cells in the starch-free area, so that the position to be occupied by the wound cork can be traced entirely across the section. In about 3 days the cork layer is pronounced, being from 2 to 4 cells deep, and the cell walls are considerably thickened. These changes are still more pronounced from the fourth to the sixth days. By the sixth day there is a continuous layer of cork, varying from 2 to 6 cells in thickness and lying from 2 to 12 cells beneath the wounded surface. Between the cork periderm and the layer comprising the surface cells is a third layer from 2 to 4 cells in depth which contains little or no starch and whose walls are considerably thickened and suberized, as shown by the fact that they stain deeply with iodin green. The thickening of the cell walls becomes evident about the third day. The cells immediately below the cork meristem merge into the cells containing the normal amount of starch.

It was found by the writers that the rate of cork development is retarded at the bottom of wounds made by striking sweet potatoes against the sharp edge of a wire basket. Coutant (*2*)[1] also found that the

[1] Reference is made by number (italic) to "Literature cited," p. 647.

cork meristem in the base of a V-shaped cut was undeveloped in the 4-day-old sections. This is thought to be due to a lack of available oxygen or to the lessening of transpiration. This latter explanation is suggested by Küster (7), who showed that at least a small amount of transpiration is necessary for wound-cork formation.

EXPERIMENTAL METHODS

The sweet potatoes used in these experiments were of the Yellow Jersey variety, grown on the Potomac flats near Arlington, Va. The first samples from which the sections were cut for the determination of wound-cork formation were taken from potatoes while still in the ground. After the potatoes were put in storage samples were taken and studied at different times throughout the storage period. For the most part the potatoes were halved and blocks of tissue about 0.5 by 0.5 by 2 cm. were removed from the cut surface in such a manner that sections made across the short diameter would be perpendicular to this surface. A control sample was taken when the experiment was started, and others were taken at stated intervals of time thereafter. The samples were dropped at once into a fixer made by adding 2 cc. of commercial formalin to 100 cc. of 70 per cent alcohol. After fixing for 24 hours or longer the specimens were dehydrated with alcohol and embedded in paraffin. Sections were cut for the most part 10 μ in thickness, stained with a weak solution of iodin green in 70 per cent alcohol, ammoniacal gentian violet, or in Pianeze IIIb stain, and mounted in balsam.

In order to determine whether wound cork is produced as readily at the center of the sweet potato as it is near the vascular ring, samples were taken from both regions. A study of sections from these regions shows that although a normal cork layer is formed in both cases, it is produced more quickly in the vicinity of the vascular ring. In the latter case the cork layer laid down in two days is to all appearances equal to that formed at the center of the potato in three days. A somewhat different condition was found in the Irish potato (*9*, *12*), where periderm formation in the seed pieces is usually not so well developed in the central as in the peripheral portion.

CONDITIONS AFFECTING CORK FORMATION

Factors influencing wound-cork formation have been considered by numerous writers, who agree that a certain amount of moisture is necessary. When the cut surfaces are allowed to dry too rapidly, a desiccation and death of the surface cells soon takes place. This results in the formation of a hard surface layer beneath which cork periderm seldom forms. On the other hand, when the escape of water from the wounded surface is retarded, a cork layer usually develops beneath the injured surface. The degree of humidity present in the ordinary moist chamber is such that cork is produced by the sweet potato. Halves of sweet potatoes

held in moist chambers healed in a normal manner, while corresponding halves kept on the desk beside the moist chamber showed only a drying and death of the surface several cells in depth. It is not known why the cork layer failed to form in the latter case. However, it may possibly be due to insufficient transpiration or to the lack of oxygen. The dry and hard surface layer no doubt serves to check the interchange of moisture and oxygen between the underlying cells and the outside air. Küster (*8*) says in this connection that at least a small degree of transpiration must be possible for the exposed tissue, while he, as well as others (*6*, *10*, *1*, *4*), regards oxygen as a determining factor. Some attention has been given by the writers to the progress of healing under storage-house conditions. Experiments were conducted to show whether cork was formed under storage conditions and, if so, what effect the temperature maintained in the house at the time the potatoes were placed in storage might play. It is generally recommended that the storage house be kept at a temperature of 25° to 30° C. during the digging period and for 10 to 15 days thereafter. Sometimes no attempt is made to raise the temperature to that height until after the potatoes are all dug. This may mean the lapse of a week or more before the curing period starts.

A quantity of sweet potatoes were taken from the field to the storage house, cut into two parts longitudinally, and samples taken each day for six days. These showed a dying of the cells for a considerable depth beneath the wounded surface but no differentiation of a starch layer and no cork formation. In another experiment the potatoes were cut on the day the curing period began. Here again there was no differentiation of a starch or a cork layer but a dying of the exposed cells, which formed a hard covering over the wounded surface. No doubt either the temperature or the humidity or both were not such as to permit the normal healing process to take place.

The potatoes used in both of these experiments showed no signs of decay in the two weeks during which they were under observation. The dried surface of the wound seemed to afford an efficient barrier to infection. In order to test further the ability of a dried surface to prevent infection the following experiments were conducted.

Potatoes were taken directly from the field and placed in a dryer designed primarily for desiccating sweet potatoes and other vegetables. The potatoes were spread out thin over a wire netting, and a current of warm air was forced up among them. The quantity of potatoes used, together with the air temperature and rate of air movement, is given in Table I. The potatoes after being treated were kept in the drying room in crates until the curing period was over, after which they were moved to the sweet-potato storage house. Observations on the keeping qualities of these potatoes were made from time to time, and samples were taken for the study of the cork formation. The table shows also the

amount of decay in each crate and the quality of the potatoes which did not rot.

TABLE I.—*Effect of drying sweet potatoes in a current of warm air upon their keeping qualities*

TREATED

Quantity of potatoes.	Time of drying.	Rate of air movement in feet per minute.	Air temperature.	Percentage decayed after 3 months.	Quality of potatoes.
Pounds.			°C.		
44½	10 minute	150 to 200	46 to 49	0	Good.
41½	20 minutes	150 to 200	46 to 49	0	Do.
50	30 minutes	150 to 200	46 to 49	0	Do.
42	40 minutes	150 to 200	46 to 49	0	Do.
42½[a]	2½ hours	150	51.5	[b]99	None marketable.
46	2½ hours	150	51.5	46	Poor; few if any marketable.
43¾	3½ hours	150	51.5	63	Do.
41	4½ hours	150	51.5	22	Poor; less than one-half marketable.
43½	9 hours	190	46 to 70	59	Very poor; none marketable.
41½	9 hours	170	46 to 70	52	Do.
40	9 hours	174	46 to 70	38	Do.
42	9 hours	190	46 to 70	83	Do.

CONTROLS

40¼	None		Same as for others after treatment.	0	Good; all marketable.
42½	do			0	Do.
40¼	do			0	Do.

[a] Potatoes halved before treatment was started. [b] In 15 days.

These results within the limits of these experiments indicate that any attempt at case hardening or of stimulating or hastening the healing of the wounds of sweet potatoes at digging time by subjecting them to a current of warm air is likely to prove detrimental rather than beneficial. The potatoes subjected to a temperature of 46° to 49° C. for 40 minutes or less were not injured appreciably, neither was their keeping quality improved. The control potatoes dug at the same time and handled in the same manner, except that they were not subjected to a current of warm air, kept equally well. On the other hand, the application of a warm air current for more than 40 minutes predisposed the potatoes to decay. It is interesting to note also that the potatoes cut in two longitudinally and then subjected to a temperature of about 51.5° for 2½ hours began to decay almost at once. Sections of some of these potatoes revealed the fact that no differentiation into a starchless layer or the formation of wound cork had taken place.

A study of the influence of temperature upon cork formation was made after the potatoes had been in storage about 2 months. Halves of potatoes from the storage house were placed in a series of chambers held at constant temperatures and humidities. Samples were taken whenever possible at intervals of 2 days for a period of 19 days. The potatoes at the higher temperatures soon decayed, so that in some cases only a few samples were obtained. Nevertheless, judging from previous work, it is thought that sufficient samples were available in every case for the purpose desired. In these tests all samples from each temperature were taken from the same half of the same potato and from a position just within the fibro-vascular ring. The temperatures and relative humidities at which these tests were carried out, as well as the results obtained, are shown in Table II. It is evident from this table that periderm formation was most rapid at a temperature of 33° C., cork production being evident in 4 days and pronounced in 8 days. The rate of cork development was about the same at 31° and 26°, producing in 11 days a layer about equal to that formed at 33° in 8 days. At 19.5° cork formation was greatly retarded, and at 11.2° no cork was formed by the fifteenth day, after which no examinations were made. Below this temperature no cork was formed within the time limit of this experiment (19 days). At 39° the potatoes decayed so rapidly that no samples were obtained after 4 days. In none of these samples was there any starchless layer formed. The walls of the cells just outside of the region where the cork layer probably would have developed if conditions had been favorable were usually somewhat thickened and suberized. The surface and subjacent cells were always dead and brown to a varying depth, depending upon the conditions of the environment.

TABLE II.—*Effect of temperature upon periderm formation*

Temperature.	Relative humidity.	Condition after healing—					
		4 days.	6 days.	8 days.	11 days.	15 days.	19 days.
°C							
44	95	(*)	(*)	.....	.....	.....	.....
39	96	(*)	.....	.....	.....	.....	.....
33	96	(†)	.....	(‡)	.....	.....	.....
31	96	.....	.....	(‡)	(‡)	.....	.....
26	96	.....	.....	(†)	(‡)	.....	.....
19.5	95	.....	.....	.....	(†)	.....	.....
11.2	97	.....	.....	.....	.....	(*)	.....
8.5	96	.....	.....	.....	.....	(*)	.....
6.8	96	.....	.....	.....	(*)	.....	.....
1	95	.....	.....	.....	.....	.....	(*)

* No cork layer. † First indications of cork layer. ‡ Cork layer well developed.

This experiment shows that a protective cork layer will form at temperatures between 26° and 33° C., while below 26° the process is greatly retarded. As stated above, sweet potatoes are cured for from

10 days to 2 weeks when first placed in storage by subjecting them to a temperature of about 25° to 30°. This is likewise the most favorable temperature for periderm formation. Experiments previously recorded showed that in those particular instances no cork was formed preceding or during the curing period. In all probability the relative humidity was the inhibiting factor. Similar experiments conducted at intervals of about 2 months throughout the season showed that at no time were the potatoes able to form a cork layer over a cut surface under storage-house conditions. However, controls held in moist chambers in the laboratory at the same time never failed to do so.

Although no periderm formed beneath cut surfaces under storage-house conditions, a cork layer was laid down just beneath dead rootlets. Likewise a similar layer of cells was laid down just beneath the wound made by breaking the potato from the roots. In the former case the dead rootlets probably served to prevent a too rapid drying of the wound, and a cork layer was formed. Likewise the deposit of the latex over the wounded end of a freshly dug sweet potato probably forms a protection against the escape of moisture, which may explain in part at least the formation of a cork layer under such conditions.

Sweet potatoes which have sprouted either in the hotbed or in the storage house have the power to form a cork layer when placed under suitable conditions. Some sweet potatoes with soft and flabby ends were placed in moist chambers in an attempt to learn whether they would form a cork layer. In most cases, however, the exposed tissues at the dried end soon turned brown and began to decay. This would seem to indicate that sweet potatoes lose their power to form a protective covering over their wounds when they once become badly shriveled, the degree of drying being the deciding factor. It has been demonstrated also (*12*) that sprouted Irish potatoes form a new cork over wounds, but that drying of the tissues, which usually accompanies germination when potatoes are stored in a warm room for a long time, retarded it.

The skin of the sweet potato, as well as that of the Irish potato (*11*), serves as an effective protection against the death of the tissues beneath by desiccation and from the attack of microorganisms. When this is broken the exposed cells would soon die were it not for their power to form some sort of protective covering. Incidentally this protective layer may possibly, at least to some extent, serve to prevent infection by microorganisms.

EFFICIENCY OF THE WOUND CORK IN PREVENTING INFECTION

The writers have nearly always failed to produce infection in sweet potatoes by Rhizopus by smearing spores and hyphae over a freshly cut surface. Nevertheless, wounded potatoes subjected to certain condi-

tions will almost invariably decay by this organism. It was thought that perhaps these studies might show some correlation between the cork formation and the susceptibility of the potatoes to decay. Consequently, experiments were designed to test the resistance of the potato to infection through wounds which had been allowed to heal for different lengths of time. In these experiments potatoes were cut in two parts and placed in a moist chamber. After they had healed for a definite length of time a fresh cut was made in one half, which was to serve as a control. Both halves were then inoculated, and the results were noted. The "well" method described by Harter, Weimer, and Adams (*3*) was used in most of the experiments described below. In these cases a hole was made into the cut half of the potato with a cork borer at the time the experiment was started and a similar hole was made in the control half on the day the inoculations were made. In other cases the method was slightly modified, in that a hole was made in the cut surface of the potato with a knife and a similar hole in the control later. In both of these cases the inoculations were made by pouring into the cavities a 24-hour-old culture of *Rhizopus tritici* in about 5 cc. of sweet-potato decoction. About half of the mycelial growth formed by this organism growing on sweet-potato decoction in a 150-cc. Erlenmeyer flask was placed in some instances upon the flat cut surface of one half of a potato which had healed for a certain number of days and the remainder on the other half, the control, from which a slice about ½ cm. thick parallel to the wounded surface was first removed.

In general, the results shown in Table III indicate that the cork layer does possess some power to prevent infection by Rhizopus, but in the majority of cases under the conditions of these experiments infection is only retarded. The method used is subject to criticism in that the test was an extreme one, since a large amount of very active hyphae and enzym was placed in contact with the healed surfaces. Such conditions would never be duplicated in nature, and probably the cork layer would never be required to withstand such a rigid test under natural conditions. However, as pointed out above, attempts to infect even a freshly cut potato with dry spores or hyphae almost always fail. It was necessary, therefore, to use some method whereby infection of the control could be obtained with certainty.

The last column in the table shows those instances where a cork layer was actually demonstrated to be present by free-hand sections.

It was pointed out above that a cork layer did not form so readily about the base of a wound made by striking a potato against a dull instrument. Although no attempt was made to determine the rate of cork formation about the base of the "well," it seems reasonable to suspect that it might not develop at a uniform rate in all places. In that case these tests of course would not necessarily indicate the true value of healing as it might

occur over a flat surface. This last criticism could not hold, however, in the cases where a piece of fungous felt was placed upon the cut surface. Here infection was retarded but not inhibited.

TABLE III.—*Effect of healing upon decay of sweet potatoes*

Date.	Number of potatoes.	Method of inoculation.	Time healed.	Results.			Presence of cork layer demonstrated.
				Control halves decayed.	Healed halves decayed.	Effect of healing.	
Nov. 17, 1919..	1	Felt placed on surface.	24 hours.	1	1	None.....	(a)
Do.....	1	.....do.....	48 hours.	1	1	Retarding decay.	(a)
Do.....	1	.....do.....	3 days...	1	1	.....do.....	Cork layer starting.
Do.....	1	.....do.....	4 days...	1	1	.....do.....	Distinct cork layer.
Do.....	1	.....do.....	7 days...	1	1	.....do.....	Do.
Do.....	1	.....do.....	11 days..	1	0	Inhibited decay.	Do.
Dec. 3, 1919....	9	Well method.	6 days...	9	9	None.....	(a)
Dec. 5, 1919....	2	.....do.....	24 hours.	2	0	Inhibited decay.	(a)
Do.....	1	.....do.....	4 days...	1	0	.....do.....	(a)
Dec. 12, 1919...	10	.....do.....	24 hours.	10	10	None.....	(a)
Jan. 22, 1920...	4	.....do.....	6 days...	4	3	Inhibited decay in 1 case.	(a)
June 17, 1920...	12	.....do.....	4 days...	12	12	Marked retarding of decay.	Distinct cork layer.
Mar. 20, 1920...	9	.....do.....	...do.....	9	9	None.....	(a)

[a] Not examined or no cork layer found.

In order to study the effect of healing upon infection when cut sweet potatoes were subjected to conditions more nearly approximating those to which they would normally be exposed, the following experiment was conducted. About ¾ bushel of medium-sized potatoes were washed with soap and water, halved, and then held for 6 days at a temperature of 30° to 32° C. and a relative humidity of about 93 as registered by a standardized Lambrecht's polymeter. The potatoes were then divided into four lots. The healed surfaces of two lots were smeared with a heavy suspension of Rhizopus spores in tap water, using a soft camel's-hair brush. The other two lots (controls) were treated in exactly the same way, except that the healed surfaces were cut away to a depth about 5 mm. before the spores were applied. One lot from each of these two sets was placed in an incubator maintained at a temperature of 25° and a relative humidity of 96. The other two lots were placed

in the sweet-potato storage house at a temperature of about 20° to 25°. Some potatoes were taken from the storage house at the time the experiment was set up, cut in halves, and the cut surfaces also smeared with the spore suspension. Table IV shows the results obtained as indicated by the final observations taken after 18 days.

TABLE IV.—*Efficiency of the healed surface in preventing decay of sweet potatoes by Rhizopus*

Treatment.	In incubator.			In storage house.		
	Number of halves decayed.	Number of halves healthy.	Percentage decayed.	Number of halves decayed.	Number of halves healthy.	Percentage decayed.
Healed	0	35	0	1	54	1.8
Healed surface removed	9	5	64	4	12	25
Freshly cut potatoes from storage house	8	6	57	16	4	80

The data contained in Table IV show that under the conditions of this experiment the healed surface formed a very efficient barrier to infection. In the incubator none of the healed potatoes decayed. On the other hand, 64 and 57 per cent, respectively, of those from which the healed surface had been removed and the potatoes which were freshly cut decayed. Somewhat similar results were obtained with potatoes held in the storage house. However, in this instance one healed specimen decayed, while 25 and 80 per cent, respectively, of the unhealed halves rotted. Since the conditions of this experiment more nearly approach those to which wounded potatoes are normally subjected, there seems to be little doubt that the healed surface forms a quite effective barrier against the attack of microorganisms. This is further substantiated by other experiments which are recorded below.

In one experiment 11 potatoes which were injured at digging time and had formed a hard protective covering over the wounds were used. A Van Tieghem cell was sealed upon the injured surface with vaseline. A 24-hour-old culture of *Rhizopus tritici* in sweet-potato decoction was poured into this cell and the top covered with a cover slip to prevent evaporation. The potatoes were held in a moist chamber at room temperature. In no case did any rot occur.

A similar experiment was conducted in which the potatoes used had been cut and held in a constant-temperature chamber at a high relative humidity for about 3 weeks. Sections through the cut surface did not show a definite cork layer, but a subserization of some of the cell walls had taken place, as was denoted by their reaction to iodin green. Four halves were used in this experiment, and in no case did any rot take place. Four other potatoes from this same lot were inoculated in the side opposite the cut surface by the ordinary well method. These were

all decayed in 5 days. The fungus, however, was not able to soften the hardened layer of tissue over the healed surface. The inability of *Rhizopus tritici* to break down this protective wound covering was demonstrated further by placing sections of this tissue in sweet-potato decoction upon which this fungus had grown for 3 days. Freshly cut sweet-potato blocks used as controls were entirely softened in less than 48 hours, whereas the tissue over the wound was not affected after 4 days.

The length of time necessary to form an impenetrable barrier over a wounded surface no doubt varies with the conditions. Data presented in Table III show that under the conditions realized in some experiments a considerable retarding effect was exhibited after 48 hours, while in other cases there seemed to be no delay or inhibition of decay after healing for 6 days.

It has been demonstrated (*1*) that a cut surface of the Irish potato healed to such an extent in 12 hours that a virulent culture of *Bacillus phytophthorus* Appel was unable to penetrate the tissue; furthermore (*5*), that wounded potatoes when inoculated 3 hours after being placed in an incubator at 30° C. failed to become infected, a protective covering having already formed.

It has also been found (*4*) that the wound cork is very effective in preventing infection of the sweet potato by *Mucor stolonifer* Ehrb. Similar results were obtained by other workers with these and other crops.

The fact that the presence of a definite cork layer could not be demonstrated in some cases where decay was inhibited or retarded would seem to indicate that this layer is not alone responsible for the protection of the wounds. Certainly no cork layer could have been laid down in the instances mentioned above, where infection of the Irish potato was inhibited by 12 hours' healing and in the case where it failed to become infected through a surface healed for only 3 hours. No doubt the suberization of the surface cell walls is a factor in preventing the entrance of microorganisms, and even the drying out of the injured cells may exert some retarding or inhibiting action against decay-producing organisms.

SUMMARY

(1) Sweet potatoes have the ability to form a cork layer over wounds when the environmental conditions are favorable.

(2) The production of a cork layer is preceded by the formation of a layer of starch-free cells, usually 3 to 10 cells deep, beneath the injured surface. Cross walls begin to appear from the second to the third day, and by the fourth to the sixth day a distinct layer of cork cells forms a covering over the wound.

(3) Temperature and humidity are important factors in regulating the formation of cork in the sweet potato. This process took place more

rapidly at 33° C. than at any other temperature tried. However, cork cells were formed at temperatures varying from 19.5° to 33°. A relative humidity of from 95 to 100 is favorable for cork production.

(4) No well-developed cork layer was produced over wounds under the conditions existing in the sweet-potato storage house. However, a dry, hard surface covering was formed through which infection by artificial means could not be obtained.

(5) The results of numerous experiments indicate that the healed surface of a wounded sweet potato forms a fairly efficient barrier against infection by microorganisms.

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TWO SCLEROTIUM DISEASES OF RICE

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SEEDLING-BLIGHT CAUSED BY SCLEROTIUM ROLFSII SACC.

In the spring of 1919 the Office of Cereal Investigations undertook a study of the rice diseases of the United States with field headquarters for the work at the Rice Experiment Station, Crowley, La., conducted cooperatively with the Louisiana Agricultural Experiment Station. On June 12 of the same year Mr. J. Mitchell Jenkins, superintendent of the Rice Experiment Station, called the writer's attention to a seedling-blight occurring in Honduras rice and resembling maggot injury. After close examination of diseased plants, no signs of the root maggot could be found, but the constant association of mycelial fibers and brown sclerotia with diseased parts was good evidence that the disease was of fungal origin. As the season advanced, the disease was found occurring to a greater or less extent in the various sowings on the Station farm. Varieties of both the long-grain and short-grain types of rice were affected. In the summer of 1920, the disease was equally as abundant on the experiment farm. It was found also on other farms near Crowley but was not so abundant. Later in the summer of 1920, while the author was at Gueydan, La., studying the "straighthead" disease of rice, the farmers seemed to be more interested in a seedling-blight which had been causing considerable damage in that vicinity. According to their descriptions of the disease it was identical with the seedling-blight at Crowley, although it was too late to obtain specimens for identification. Reinking [1] reports the occurrence of a Sclerotium disease of rice in the Philippine Islands. He states that damage is caused in the seed bed and in rice near maturity, where the stems are attacked and sterile heads result. Reinking did not attempt to place the fungus specifically, but cultures of the Philippine form obtained from Mr. G. O. Ocfemia, a Philippine student in plant pathology at the University of Wisconsin, apparently are identical morphologically with the form in Louisiana.

SYMPTOMS

The appearance of small areas of blighted or dead seedlings is the first noticeable sign of the disease in the field. (Pl. 122, A.) These areas tend to follow the drill rows, and a part or all of the seedlings may be

[1] REINKING, Otto A. PHILIPPINE ECONOMIC-PLANT DISEASES. *In* Philippine Jour. Sci. Sect. A, v. 13, no. 5, p. 228. 1918.

dead for several feet or even several yards. The disease may follow a row or extend across two or more rows, which are about 8 inches apart. The dead plants have a dark, moldy appearance, which indicates slow death and the presence of various saprophytic fungi. Alternaria and Helminthosporium are commonly present on these dead plants. Plants which are killed by maggots are usually lighter in color, due to the rapid drying after the stems are severed from the roots. The base of the stem and the roots of diseased plants are dark in color and often have a frosty appearance, due to the presence of wefts of the mycelium of the causal fungus. Brown sclerotia may be found attached to the roots or the base of the stem of diseased plants. Plants attacked by this fungus are not so easily removed by pulling as are plants severed at the base by root maggots, although the roots are almost completely decayed by the time the plants are dead. Infected plants first show a stunted condition accompanied by yellowing and withering of leaves, and a high percentage of the plants may show a distinct lack of chlorophyll or "pseudo-albino" condition, which may appear as white stripes in the leaves or as almost totally white leaves. Part or all of the leaves on a plant may show this condition. Rice seedlings weakened by other root troubles in some cases may show this same lack of chlorophyll. Plants badly affected die slowly, the tips and margins of leaves dying first and thus affording a medium of early attack by saprophytic forms. Plants that survive until the irrigation water is applied, if not too nearly dead, usually recover, due to the fact that water immediately checks the development of the fungus.

IMPORTANCE OF THE DISEASE

The distribution of the disease is not known, and consequently no estimate of damage can be given. In localities where it occurs, however, considerable losses may result. Early sown rice seems more subject to attack by the fungus than that sown later. This probably is due to the fact that seed sown later germinates more readily and the plants grow more vigorously, thus giving the fungus less chance to overcome them before the irrigation period. The quantity of fungus material may be reduced somewhat because of the decrease of organic matter in the soil which serves as food for it. The fact that a large percentage of the seedlings may be destroyed before emerging from the soil makes the disease more important than might be suspected from the dead seedlings seen in the field. By digging into the soil, seedlings in various stages of development are found to have been destroyed.

Still more importance was attached to this disease when an apparently identical fungus was found growing in abundance on soybean plants on the station farm where the soybean is being considered as a desirable crop to rotate with rice. If these forms proved to be identical there would be considerable danger of disseminating the fungus with soybean seed.

CAUSAL ORGANISM

Small, brown, spherical sclerotia were found constantly associated with the decayed roots of blighted seedlings. The coarse, white mycelium typical of *Sclerotium rolfsii* was present at the base of the stems, on the diseased roots, and in the soil immediately surrounding the roots. Fragments from freshly diseased tissues, plated on sterile agar, gave pure cultures of *S. rolfsii*, as did the sclerotia which were surface-sterilized and sown on sterile media. The organism grows vigorously and produces sclerotia abundantly on boiled rice. In culture it appears slightly different morphologically from the forms occurring on soybean, wheat, and tall oat grass (*Arrhenatherum elatius* (L.) Beauv.), but more nearly like the form from wheat. The difference, however, in any case is very slight. The sclerotia of the form on rice are more uniformly spherical, the mycelium is not so coarse in appearance, and there is also a physiological difference brought out later in comparing the ability of these forms to parasitize rice. There is not enough difference, however, to warrant their separation as distinct species.

The rice fungus is not a vigorous parasite. It attacks germinating seed and young seedlings before the root system is sufficiently established to combat the invasion. It is very destructive to germinating seed but acts as a slow parasite on seedlings that have emerged, gradually destroying the tissues of the roots from the surface inward (Pl. 124) until the seedlings are dead. It kills seedlings readily when grown in tubes on nutrient agar (Pl. 123). If there is an abundance of the organism in the soil the stand of plants may be greatly reduced (Pl. 122, B). On soil inoculated by sowing sclerotia with the seed the stand was reduced as much as 50 per cent in some cases. Comparisons were made with the various strains of *Sclerotium rolfsii* mentioned above—namely, from rice, soybean, wheat, and tall oat grass. The results of these experiments, given in Tables I and II, show that the forms from rice and wheat are equally parasitic to rice, while the forms from soybean and tall oat grass did but little damage to rice. This does not mean necessarily that all cultures from the soybean and tall oat grass would be such weak parasites to rice. They also might become more parasitic after a period of adaptation accomplished through contact with the rice plant.

For experimental purposes, large galvanized cans 16 inches square and 15 inches deep were used. These were filled with about 10 inches of soil from a field where rice was never grown, obtained in such a way as to avoid contamination as much as possible. Each can had a drain pipe at the bottom for the purpose of regulating the water supply. The cans were arranged in series of 10, and soil was banked against them on the sunny side to prevent overheating and too rapid evaporation (Pl. 122, B). The soil was inoculated by sowing the fungus sclerotia with the rice seed. Honduras rice was used for these experiments, and 100 seeds were sown in each can.

TABLE I.—*Results of germination of Honduras rice in soil inoculated with Sclerotium rolfsii from rice, soybean, tall oat grass, and wheat; sowings made on May 4, 1920, and counts taken May 22*

Can No.	Source of fungus.	Treatment.	Percentage of germination.[a]	Number of plants chlorotic.	Number of plants dead before irrigation.
1	Rice	Inoculated	25	3	0
2	do	Control	**71**	0	0
3	do	Inoculated	40	10	4
4	do	Control	**55**	0	0
5	do	Inoculated	37	5	3
6	do	do	28	5	2
7	do	do	24	4	1
8	do	do	33	3	2
9	do	do	33	7	5
10	do	do	44	10	4
1	Tall oat grass	do	79	2	1
2	do	do	71	3	0
3	do	do	70	7	0
4	do	do	63	2	0
5	do	do	78	8	0
6	do	do	58	3	1
7	do	do	66	3	0
8	do	do	63	0	1
9	do	Control	**73**	1	0
10	do	do	**71**	0	1
1	Soybean	Inoculated	57	0	1
2	do	do	55	0	0
3	do	do	59	1	4
4	do	do	48	3	0
5	do	do	62	1	1
6	do	do	63	1	0
7	do	do	58	0	0
8	do	do	69	0	0
9	do	Control	**71**	0	0
10	do	do	**72**	0	0
1	Wheat	Inoculated	34	5	0
2	do	do	36	10	0
3	do	do	34	4	0
4	do	do	34	3	1
5	do	do	23	4	0
6	do	do	18	3	1
7	do	do	35	5	0
8	do	do	25	9	1
9	do	Control	**74**	0	0
10	do	do	**59**	0	0

[a] Controls are given in boldface type.

Table I shows that the percentage of rice germination was considerably reduced where the soil was inoculated with *Sclerotium rolfsii* from rice and wheat, while strains of the organism from soybean and tall oat had but little effect on germination, although some of the seedlings were diseased. The average gemination in soil inoculated with the rice fungus was 33 per cent, as compared with 63 per cent in uninoculated soil. There was an even more striking difference where the soil was inocu-

lated with sclerotia from the wheat fungus. Here the percentage of germination in inoculated soil was only 29.9, as compared with 66.3 in uninoculated soil. There was a germination of 59 and 71.5 per cent, respectively, in soil inoculated with the soybean fungus and in uninoculated soil, and a germination of 68.5 and 72 per cent, respectively, in inoculated and uninoculated soil where the tall oat grass form was used. The difference in the case of the tall oat grass form could easily have been a variation in individual controls, as some of them ran as low as 63 per cent. There was a noticeable reduction, however, where the soybean fungus was used as the inoculum. Diseased plants in these experiments continued dying until the irrigation water was applied, after which time all plants recovered, if not too severely damaged. The high percentage of chlorotic or pseudo-albino plants was very striking where the disease was present. A high percentage of these chlorotic seedlings died before irrigation.

TABLE II.—*Results of germination of Honduras rice in soil resown on June 29, 1920, after removal of a crop 7 weeks old*[a]

Can No.	Source of fungus.	Treatment.	Percentage of germination.	Number of plants chlorotic.	Number of plants dead before irrigation.
3	Rice	Inoculated	67	0	2
4	do	do	59	0	0
5	do	Reinoculated	30	1	5
6	do	Inoculated	81	0	0
7	do	do	64	0	2
8	do	do	75	0	0
9	do	Control	74	0	0
3	Tall oat grass	Inoculated	75	0	0
4	do	do	61	0	0
5	do	Reinoculated	51	12	4
6	do	Inoculated	75	0	0
7	do	do	74	0	0
8	do	do	67	0	0
9	do	Control	74	0	[b] 1
3	Soy bean	Inoculated	74	0	0
4	do	do	75	0	0
5	do	Reinoculated	70	7	2
6	do	Inoculated	64	0	0
7	do	do	74	0	1
8	do	do	59	0	0
9	do	Control	70	0	0
3	Wheat	Inoculated	60	0	1
4	do	do	80	0	3
5	do	Reinoculated	41	5	8
6	do	Inoculated	61	0	0
7	do	do	76	0	2
8	do	do	64	0	0
9	do	Control	68	0	0

[a] At the time of the first seeding the soil was inoculated with *Sclerotium rolfsii* from rice, soybean, tall oat grass, and wheat. Can No. 5 in each series was reinoculated.
[b] Worm injury.

Two inoculated cans and one control can from each series were irrigated at a depth of 4 to 5 inches 20 days after planting, and none of the plants died after the irrigation water was applied. Plants which showed signs of the disease revived rapidly after the application of the water. About 7 weeks after sowing the plants were removed from the remaining seven cans in each series, and the cans were resown. The Honduras variety of rice was used, as in previous experiments, and 100 seeds were sown in each can. Can No. 5 in each series was reinoculated with fresh cultures of the same organisms used with the previous sowings. Results of these experiments are given in Table II.

The results given in Table II indicate that the fungus is practically exhausted after eight weeks in the soil where rice is grown. Soil that was reinoculated (can No. 5) in each case showed the same proportionate decrease in germination as was shown in Table I.

ENDURANCE OF THE FUNGUS

The fungus remains in the sclerotial stage during seasons that are unfavorable to growth. The mycelium will live for considerable lengths of time in the infected plant tissues and in the soil where there is sufficient organic matter to furnish food. Sclerotia kept in a dry condition for nine months germinated readily when placed under proper conditions of moisture and temperature. On May 28, 1920, sclerotia of the fungus were placed in small vials with cheesecloth over the top and immersed in water in the laboratory and in the field where rice was irrigated. On September 10 these sclerotia were almost 100 per cent viable. They were still viable on November 4. Under field conditions where the disease occurred the fungus lived through the period of irrigation and started developing on the roots and stems of plants after the fields were drained.

DISSEMINATION

The sclerotia float on the surface of water like cork and may be carried easily by irrigation water. The fungus also grows vigorously as a saprophyte and may be carried about on old rice straw or in hay or other plant material that is moved from fields where it occurs.

CONTROL MEASURES

Thorough cultivation hastens the decay of organic matter in the soil and also favors the germination of the sclerotia of the fungus. If infested soil is plowed early, allowed to stand for a few weeks, and then disked or well harrowed before seeding, the organic matter will have a chance to decay and the fungus will be more or less exhausted. Rice sown later than the average germinates readily and grows vigorously, thus giving the fungus less chance for attacking it. This is especially true where the soil is well prepared and a good seed bed is made. Seed sown before the soil has become thoroughly warm does not germinate promptly, and the growth of the seedlings is slow. Seedlings in such a

weakened or slow-growing condition are very susceptible to attacks by parasitic organisms, a fact which probably accounts for the higher percentage of the disease in early sowings.

The fungus requires considerable air for its growth and consequently does more damage to rice seedlings when the soil is rather dry. Reinking [1] states that—

> When there is a lack of water in seed beds, the disease appears to be at its worst. Seed beds should be kept flooded.

When the land is irrigated the growth of the fungus is checked and no further damage is caused, while all plants recover that are not too nearly dead. If the disease begins to develop, the field should be irrigated as soon as possible. Observations, both in the field and in sowings made in inoculated soil, reveal the fact that the disease is completely checked by water. If the soil of a field is known to be infested, a much better stand may be obtained by irrigating the land sufficiently to wet it thoroughly at the time the seed is germinating. This would tend to hold the fungus in check at the most critical stage in the seedling growth, as shown in Tables I and II. These experiments showed that the greatest injury was done before the seedlings emerged from the soil.

To control the spread of the disease the rice grower should prevent irrigation water from flowing from infested fields to clean fields and avoid taking rice straw or hay from fields where the disease occurs and putting it on other soil, as the organism may be growing saprophytically on such dead material. Although the fungus from the soybean proved much less parasitic on rice than did the rice form, it is still possible for the rice fungus to be carried on the the soybean plants. It may also be carried as a saprophyte, and it is advisable to select clean bean seed, especially if it is to be sown on uninfested land.

STEMROT CAUSED BY SCLEROTIUM ORYZAE CATT.

In the rice fields at Crowley, La., in the spring of 1919, an abundance of small, black sclerotia were found in the tissues and on the surface of old rice plants and stubble of the previous year. The same condition was noted again in the spring of 1920, at which time the organism was obtained in pure culture from the sclerotia. The fungus was then thought to be of an entirely saprophytic nature. On August 5, 1920, considerable lodging was noted in a field of Early Prolific rice which was being harvested near Crowley. The affected plants were more or less collapsed, and the panicles were generally poorly filled and light. Near the ground, two or three nodes from the base, the stems appeared darker, and the internode where the irrigation water stood was found to be almost completely destroyed (Pl. 125). Only the thin epidermal layer remained intact, and the stem often was completely collapsed at this point. In the cavity of the diseased portion of the stem and in the diseased tissues

[1] REINKING, Otto A. PHILIPPINE ECONOMIC-PLANT DISEASES. *In* Philippine Jour. Sci. Sect. A, v. 13, no. 5, p. 228. 1918.

a weft of fine, white mycelium and numerous small spherical sclerotia could be seen (Pl. 126). Sclerotia were noted also in the sheath tissues. Apparently the sheath is the first part of the plant to be attacked.

Indications are that the sclerotia float on the water and thus come in contact with the leaf sheath, where they germinate and penetrate the tissues, causing dark brown areas. These lower sheaths die when submerged in the irrigation water, and the fungus lives more or less as a saprophyte in the dead tissues and finally attacks the stems after the plants are older. The most severe damage is caused at the time the vitality of the stem is declining and the panicles are filling. The plants are weakened, and the resulting panicles are poorly filled and light.

The fungus is easily cultured from sclerotia by dipping them in alcohol and then in mercuric chlorid (1 to 1,000) for five minutes, washing in sterile water, and plating on agar. Fragment cultures from invaded tissues also are successful. The organism grows vigorously on boiled rice and produces an abundance of the small black sclerotia. The mycelium, which is normally white, becomes a dark smoky color at the surface of the medium. The hyphae are 3 to 5 microns in diameter, septate, and profusely branched. Dry, mature sclerotia from the diseased stems range in size from 220 to 270 microns in diameter, averaging about 250 microns. They are black, and rather uniformly spherical, with a smooth surface.

Cattaneo [1] described the organism in Italy in 1879 as follows:

> Sclerotia black, 1/10 mm. in diameter, glistening, arising from a slendor white mycelium.

The measurements of sclerotia given by Cattaneo are smaller than sclerotial measurements of the form found in Louisiana, but in all probability his measurements were roughly made. Shaw [2] gives a range of measurements of the sclerotia of *Sclerotium oryzae* which easily covers the dimensions of the American form. Shaw measured sclerotia from cultures, however, and this probably accounts for the wider variation in size. His description of the fungus on glucose agar is as follows:

> The sclerotia are at first visible as minute circular spots of a grayish color; subsequently they become black and shiny, exactly resembling those found in the rice plant. The hyphae are of the usual type, the cells being about 4 to 6 microns broad and 150 to 350 microns long. They contain numerous oil globules and frequently branch. A tranverse septum occurs at the point of origin of a branch and not some distance from it. The sclerotia are roughly circular and vary in diameter from 150 to 500 microns. They arise from a plexus of interlacing hyphae which continue to branch and intertwine until a small, spherical mass is formed. For a time the young sclerotium increases in size by the adhesion of fresh branches to the periphery; ultimately the cell walls turn black and all further growth ceases.

Symptoms of the disease given by the eastern writers are identical with the symptoms of diseased plants in Louisiana with the exception of tiller-

[1] CATTANEO, Achille. SULLO SCLEROTIUM ORYZAE, NUOVO PARASSITA VEGETALE . . . *In* Arch. Lab. Bot. Crittogamico R. Univ. Pavia, v. 2/3, p. 75-83, pl. 7. 1879. Bibliografia del genere Sclerotium, p. 81-83.

[2] SHAW, F. J. F. A SCLEROTIAL DISEASE OF RICE. *In* India Dept. Agr. Mem. Bot. ser., v. 6, no. 2, p. 11-23, 3 pl. 1913. Bibliography, p. 21-22.

ing, mentioned by Shaw [1] and Butler [2] in India. This condition does not occur in this country to any noticeable extent and is not mentioned by Miyake [3] in Japan. This perhaps is due to earlier attacks by the fungus in India, which cause the injured plant to produce tillers. All other evidence indicates that the disease in the various countries is caused by *Sclerotium oryzae*. The fungus was probably introduced into America with seed rice from some of the oriental countries.

Honduras rice plants were inoculated by inserting mycelium of the fungus into needle punctures in the stem tissues near the water surface. Within 11 days there were noticeable lesions, and one plant had fallen from stemrot. One month after inoculation all plants were diseased, and sclerotia were present in the diseased tissues of some of them. Shaw [1] reports 70 to 80 per cent of fatalities to rice seedlings grown on nutrient agar and inoculated with the fungus.

In 1920, the disease did considerable damage to early rice in the vicinity of Crowley, La., where it was first noted in this country. Early Prolific rice is especially susceptible to its attack, but all varieties were more or less affected. The disease was found also at Elton, La., where it did noticeable damage in 1920. Reports of its occurrence in Arkansas have been received, but specimens have not been obtained for identification. Cattaneo [4] states that the disease was destructive in Italy as early as 1879. Miyake [3] reports its occurrence in Japan in 1910 and says great damage is caused to the rice crop where diseased. Shaw [1] and Butler [2] in 1913 report considerable damage in various localities in India due to this disease.

During the winter and other periods unfavorable to growth the fungus survives in the sclerotial stage. The sclerotia from old rice straw and stubble germinate readily in the spring. These sclerotia can be carried easily from field to field by the irrigation water on which they float. Straw removed from the diseased areas also will carry the fungus.

The Japanese varieties apparently are less susceptible to the disease than are the long-grain rices. Early Prolific seems to be the most susceptible of all. There is a possibility of developing a resistant variety by selecting plants which withstand the attacks of the fungus. All diseased plants should be carefully removed from plots where seed rice is to be obtained. Where the soil is infested with the fungus the more resistant varieties should be grown in preference to varieties that are known to be highly susceptible.

[1] Shaw, F. J. F. op. cit.

[2] Butler, E. J. diseases of rice. *In* Agr. Research Inst. Pusa Bul. 34, p. 34-36. 1913.

[3] Miyake, Ichiro. studien über die pilze der reispflanze in japan. *In* Jour. Col. Agr. Imp. Univ. Tokyo, v. 2, no. 4, p. 264. 1910.

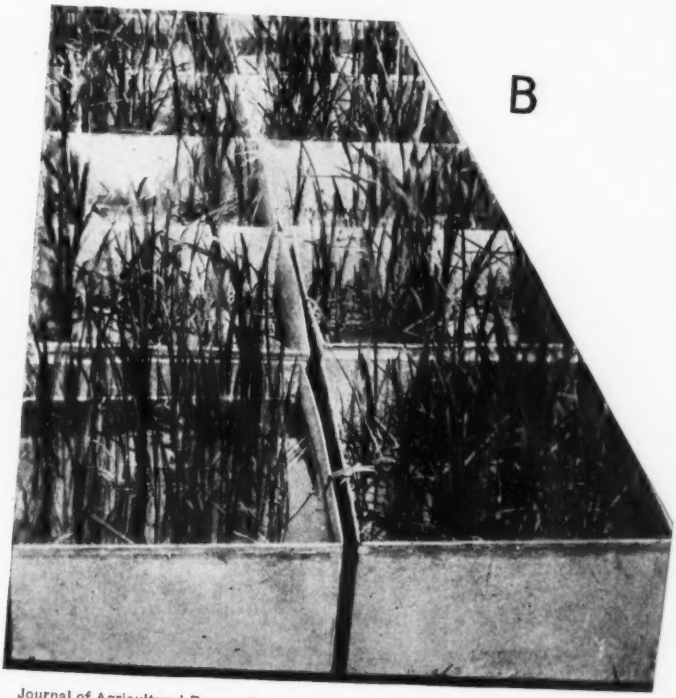
[4] Cattaneo, Achille. op. cit.

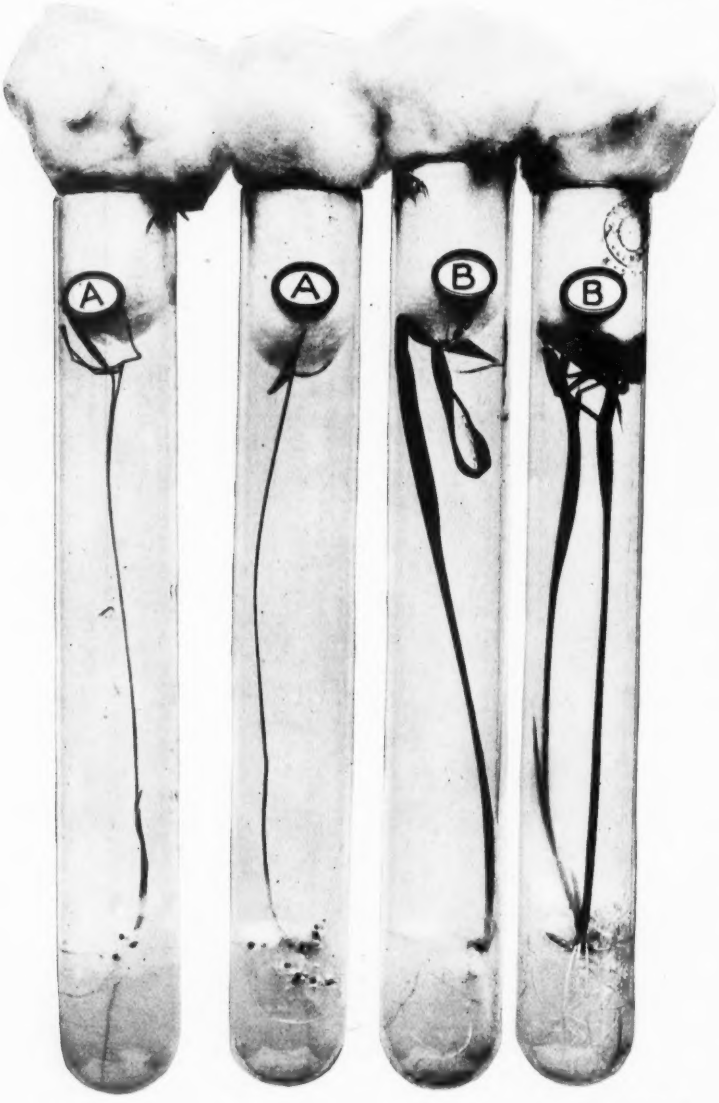
PLATE 122

A.—Field showing seedling-blight of rice caused by *Sclerotium rolfsii*. Note dead plants and lack of stand in the two rows in center.

B.—The effect on the stand of rice sown in soil inoculated with *Sclerotium rolfsii* from wheat. The first two cans in the series are uninoculated. Note that seedlings are much more numerous in these two cans.

A


B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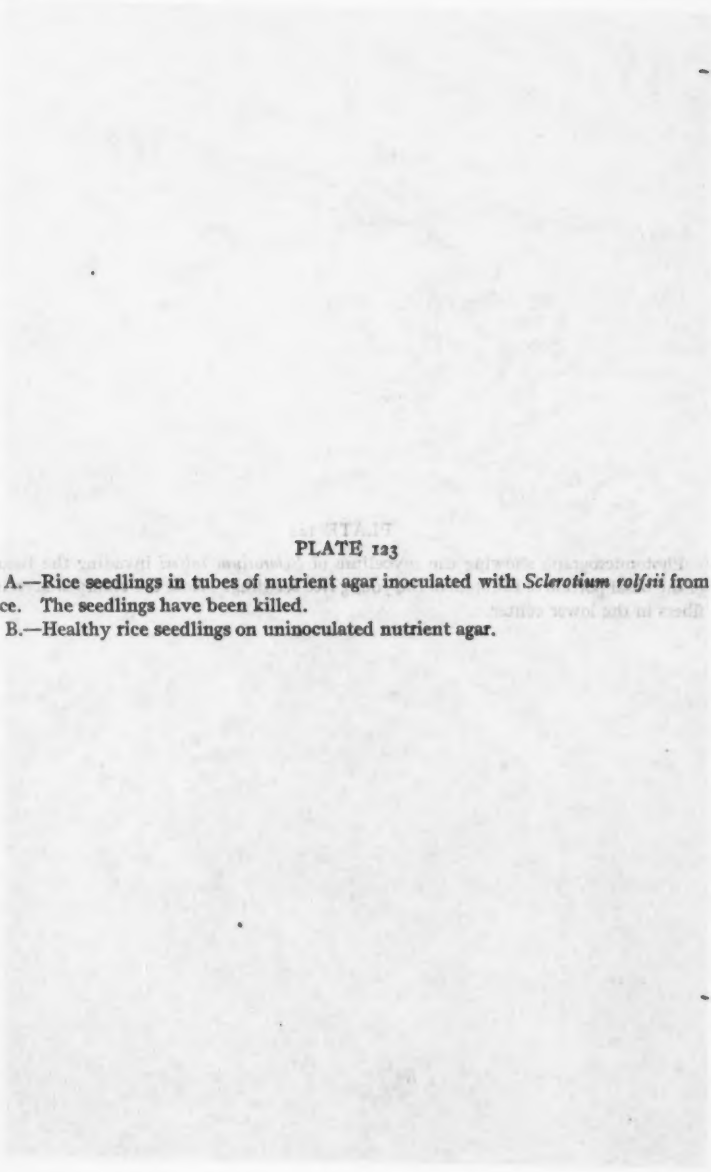


PLATE 123

A.—Rice seedlings in tubes of nutrient agar inoculated with *Sclerotium rolfsii* from rice. The seedlings have been killed.

B.—Healthy rice seedlings on uninoculated nutrient agar.

PLATE 124

Photomicrograph showing the mycelium of *Sclerotium rolfsii* invading the tissues of the basal portion of the stem of the young rice seedling. Note the clump of mycelial fibers in the lower center.

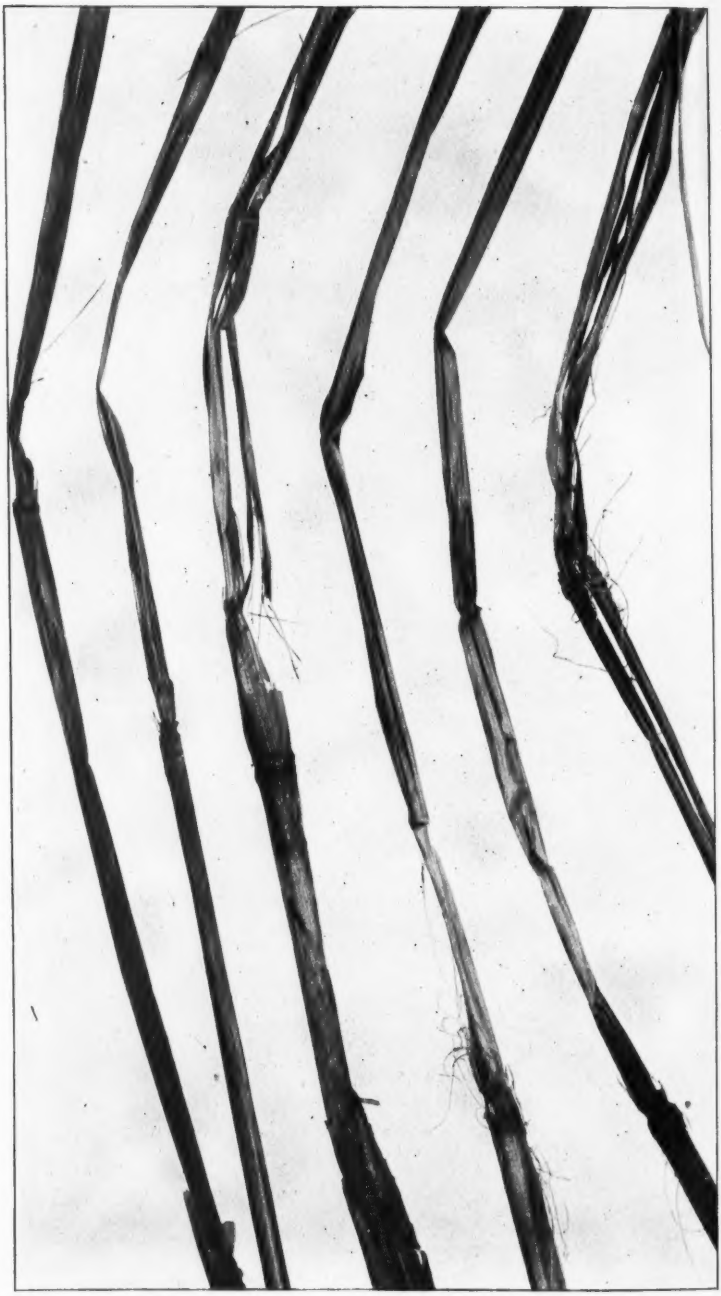
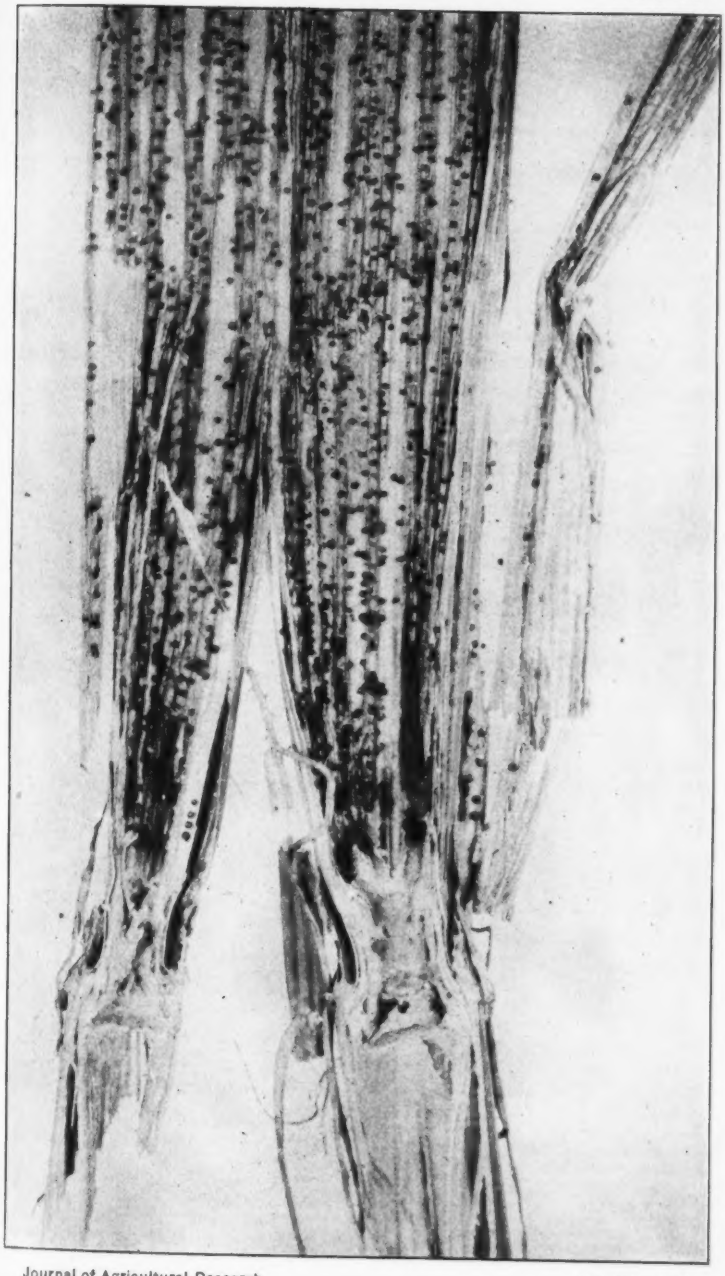


PLATE 125

Stems of Early Prolific rice attacked by *Sclerotium oryzae* Catt. Note that the internodes are completely collapsed.

PLATE 126

Enlarged photograph showing the destroyed inner portion of a rice stem attacked by *Sclerotium oryzae* and the numerous black sclerotia of the organism present.



A BIOLOGICAL STUDY OF THE RED DATE-PALM SCALE, PHOENICOCOCCUS MARLATTI

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INTRODUCTION

The present paper gives the results of a study of the biology of the red date-palm scale made during monthly field inspections in the Coachella Valley, Calif., during the year 1920. This study was conducted with the object of determining the factors in the life history which may be of importance in considering control of this pest. No attempt has been made to determine the details of the life cycle, such as length of instars, variation in length of life cycle, etc., excepting those more important points which bear directly on the general study. Most of the work was conducted at the Government Gardens at Mecca, Calif., as the older trees in these gardens are generally heavily infested with scales, but frequent inspections were made of several of the larger commercial plantings to check up the observations.

DISTRIBUTION

This scale was discovered in 1890 by Dr. C. L. Marlatt at Washington, D. C., on some date palms imported from Algeria, North Africa. In 1899 Mr. T. D. A. Cockerell, while in Washington, studied the material collected by Dr. Marlatt and described the insect as a new genus and species (*Phoenicococcus marlatti* Ckll.). Several years later Mr. Cockerell found small colonies in palms in the gardens at Tempe, Ariz. Since then the infestations have increased, and it has been brought in with practically all of the later introductions of offshoots into California and elsewhere. Distribution of these and other infested offshoots has established the scale in practically every garden of imported palms.

There are over 10,000 imported palms in orchard form in the Coachella Valley now, and practically all are infested.

The quarantine act of 1913[2] affecting interstate movement of infested palms has restricted the distribution of this insect outside of the States

[1] The writer wishes to acknowledge the assistance of Mr. Bruce Drummond and Mr. A. J. Shamblin, of Indio, Calif., who have given freely of their time and practical field experience. In many ways the present work has been corroborated by the observations and experiences of these men.

[2] [AN] ACT TO REGULATE THE IMPORTATION OF NURSERY STOCK AND OTHER PLANTS AND PLANT PRODUCTS; TO ENABLE THE SECRETARY OF AGRICULTURE TO ESTABLISH AND MAINTAIN QUARANTINE DISTRICTS FOR PLANT DISEASES AND INSECT PESTS. ... [Approved August 30, 1912, amended March 4, 1913, and March 4, 1917. *In* U. S. Statutes at Large, v. 37, pt. 1, p. 315-319, 853-854, 1913; v. 39, pt. 1, p. 1165-1166, 1917.

and counties then known as infested, but distribution within this area has been only partially retarded.

There is little probability of the dissemination of the scale by its own movement, as only during its very young stage does it move at all and it could hardly travel from tree to tree at this time.

The most important means of distribution is through the infested offshoots. These offshoots are invariably rather heavily infested and derive the infestation from the bole of the parent tree. Many severely infested offshoots have been found in the propagation houses, where they are placed for rooting.

Of lesser importance is the accidental carriage of the scale by the men at pollination time; and carriage by birds, insects, and the wind, especially in the spring during the so-called migratory period of this scale insect.

ECONOMIC IMPORTANCE

In severe infestations this scale has the habit of massing on the new succulent fruit stems and leaf bases of the palm in such numbers as greatly to weaken their normal development. In fact when the fruit stems are severely attacked the flow of sap is so greatly arrested that the fruit drops just before reaching maturity. Mr. Drummond states that a heavily infested Tazizaoot palm lost its entire crop for three successive seasons at the Mecca gardens. One date-growing company lost approximately 1,200 pounds of fruit from 75 infested palms in 1917. In the early spring during the migratory period the insects mass not only on the fronds but also on the fruit clusters and occasion serious damage. That they also retard the normal growth of the palm is evident.

DESCRIPTION OF STAGES.

In the body of the mature, wine-colored insect may be seen the developing embryos with their six legs, antennæ, dark eye spots, and coiled mouthparts. The young are born alive and issue from the ventral side of the mother scale into a depression in the body wall.

LARVA

The young female larva is flat and oval in shape, pinkish white in color, 0.24 mm. in length, with body segmentation fairly well defined. The antennæ are 6-segmented, the basal segment being very broad and the terminal segment cylindrical. The abdomen is 7-segmented and bears two pairs of setæ on the caudal end, the inner pair being nearly twice as long as the outer. The mouthparts are fully twice the length of the body, exceedingly fine and whiplike.

After remaining under the mother scale for a time the larva crawls out and wanders about in search of a suitable place to feed and then settles for life. The long stylet mouthparts are inserted, and the larva begins

to feed. The body becomes greatly distended with sap and assumes a rounded elliptical shape, rather shiny and yellowish white in color. Fine filaments of "cotton" are given off, first from the lateral glands, and before long the entire body is covered with a cottony mass.

Under this mass the first molt occurs. The cast skin shows a ventral split and the exuvia is thrown off dorsad of the new insect (the mouth-parts remaining attached) and is incorporated in the posterior end of the cottony mass.

The larva now appears somewhat long, oval, light yellow in color, without legs, and with antennæ reduced to small tubercles. Feeding continues and additional "cotton" is secreted, but the insect enlarges so rapidly as to split the cottony mass dorsally and expose the insect. The skin becomes very tight before the second molt.

The second molt is now passed, the cast skin splitting on the ventral side but not always completely thrown off, and the rapidly growing insect (now an immature adult) spreads it so as to leave it incorporated on the upper edges of the old "cotton." The new insect is nearly round but somewhat flattened, 1 to 1.25 mm. in length, wine-red in color, and without apparent appendages or segmentation, but the antennæ under high power are still seen as minute tubercles. The cottony mass formed during the immature period forms a nestlike bed for the scale. Embryos are soon formed within the body and develop rapidly. With the development of the mature scale the wine-red is replaced by a light brown, which starts at the margins; and the color changes to a bronze in the old, dead scale. No embryos have been found in the bronze-colored scale. From field observations it was found that in the cooler part of the year the insect passes from larva to a fully matured adult in approximately a month, and doubtless in the warmer months this period is greatly shortened. As to the life of the scale no definite records have been taken, but field observations would indicate that the scale lives from six to nine months. This is determined by the development of the scale on the fruit stems and leaf bases which become infested in May but have no dead scale on them until late in November.

MALE

Thus far the writer has been unable to discern any distinction as to sex in the larva up to the first molt. From the first molt, however, which in the male form is similar to that described above, there comes a larva similar to but smaller than the female second stage; and from this comes the male pupa, which is rather long, yellowish, and with antennæ and legs folded close to the body. At the third molt the adult male issues, usually through the end of the cottony cocoon. It is shaped not unlike a thrips, with distinct body segmentation, somewhat club-shaped antennæ, rather stout legs, and long, narrow, pointed abdomen, but without wings. The

male, 0.4 mm. long, is very active and moves about much more quickly than the female larva. The number of adult males in proportion to adult females is exceedingly small. Throughout the year, in examining over 300 larvæ and cocoons, the writer found as many as 30 male pupæ but only 8 adult males.

NATURE OF INFESTATION.

The main infestation of scales on a mature palm (Pl. 127) is found on the white living tissues of the leaf bases and fiber bands from 1½ to 3 feet down from the crown, or from the fifth to tenth leaf whorl (Pl. 128). Both inner and outer surfaces are usually infested, and the area may be from ½ inch to 5 inches in width. The scales will be massed together, often in more or less definite order and frequently several layers deep. Infestations of lesser severity will continue on the living tissues of the old leaf bases and fiber bands even to the ground, but as the margin of living tissue narrows the quantity of living scales becomes appreciably less. These insects are usually buried to a depth of 4 or 5 inches under the plant tissues; and as the fiber bands and leaf bases are exceedingly tight, the scale is well protected from heat, dry atmosphere, or control measures. Due to the rapid growth of the palm and definite migratory habit of the scale, the crown of the tree for from three to five leaf whorls, depending upon its size and growth, is kept free of scales. This has been proved by cutting open the crowns of several palms and offshoots. During the greater part of the year (July to April) practically all of the scales are found on the leaf and fiber bases and the fruit stems. The small number found out in the leaf pinnæ are usually of little importance.

That the fruit stems of the current year are found in the area of the main infestation and are tender and succulent at their base explains in part why they are invariably so heavily infested. A number of fruit stems have been removed which were completely massed with scales ⅛ inch thick for a distance of from 6 to 18 inches up from the base. Such infestations seriously injure the development of the fruit. Another important factor is that the fruit stem spathes open and the tender young stem is exposed at the same time that the so-called "spring migratory period" of the scale takes place, thus insuring a complete infestation of that tissue. The buds for the blossoms are laid down deep under the leaf bases in July to September. The bud growth continues through the fall and winter and the spathe pushes out in the spring. Flowering occurs in early April, or late April and early May, depending upon the season and plant variation. Young palms often show greater variation. It is during April, May, and part of June that the greatest migratory period of the scale occurs and the exposed fruit stems become infested. The progress of this infestation was definitely traced during April, May, and

June of 1920. By the latter part of June the fruit stems of the current year were well infested.

Of the infestations on the roots only the so-called superficial roots have been observed by the writer to be infested (Pl. 129, 130). These short, fleshy, gnarled roots, forming above ground, at the base of the bole, are usually under decaying leaf bases and frequently, on old palms, carry a relatively light infestation. They are often so concealed as to be entirely protected. Attention was drawn to an infestation below the ground surface on the bole of a young palm planted in a commercial grove, but on examination it proved to be only a case in which an infested offshoot had been set out and planted deeper than normal, thus burying some of the scale infestation. It has been reported that the scale has been found on the main roots of the palms planted in a very heavy soil at Heber, Calif., which cracked open after each irrigation and left the roots more or less exposed, but no roots have been observed to be infested in the usual sandy soils.

Some scales may be found in the pinnæ of the leaves for a distance of from 2 to 6 feet out from the trunk at all seasons of the year, but the quantity is usually negligible. They undoubtedly get out there during the migratory periods and by the natural pushing out of the growing leaves, but usually they are almost completely controlled by the heat of summer. In some cases scales will persist under the sand deposited in the pinnæ and will reproduce, but in most instances this infestation has been almost negligible after a few hot, dry days. The greatest number are found out on the leaves from the latter part of April until the latter part of June, though they occasionally appear again in September and October during the so-called fall migratory period. The infestations in the leaf pinnæ almost always cause brown pittings on the underside of the pinnæ, and these markings are often used to determine whether a tree is infested or not when it can not be cut into. By July only a few living insects are found in the leaf pinnæ.

Certain varieties of palms show heavier infestations than others. The following generally develop heavy infestations: Horra, Tazizaoot, Rhars, and Rohm Gazal. Palms vary greatly in the tightness of their leaf fiber and bases, but this does not explain the variation in susceptibility to scale attack, as the variety Horra has a relatively tight fiber, but is possibly the most severely attacked.

SEASONAL HISTORY

The insect has been found in all stages of development at all seasons of the year. This would indicate a continual reproduction and overlapping of generations. Though possibly the greater number of active larvæ were observed from March to December, even in January the scale was in all stages of development. In January, however, the mortality among

the young larvæ, either from moisture (mildew) or cold, appeared to be higher than at other times. The greatest development of the scale is possibly reached in the spring (April to June). This is the so-called spring migratory period when the young leave the old areas of infestation and migrate up on the bole and out on the new growth of offshoots to new areas. From the old center of infestation in the area of last year's fruit stems the new center of infestation is moved to the area of the fruit stems of the current year. This is accomplished by actual migration of the young larvæ to the new leaf and fiber tissues above and to the leaf pinnæ. That by June many of these larvæ are killed in the exposed places is evident. From about the middle of April palms with heavy infestations show quantities of cottony masses out on the fronds and new fruit stems and on the new growth of their offshoots. By July this has practically all disappeared.

Mr. Shamblin has often observed a fall migration similar to the spring migration. It is much less severe and the "cotton" of the exposed insects is usually found out on the fronds of the offshoots and parent tree during September and October and disappears almost entirely by the latter part of November. The occurrence of this migration depends entirely upon weather conditions, a cool fall usually bringing on a migration. No fall migration was noted in any gardens during 1919. That this appearance of the insect is usually of relatively small proportion (less than half of the spring generation) and often is completely controlled by weather conditions makes it of much less importance than the spring occurrence.

In Tempe, Ariz., there is much less scale development than in the Coachella Valley, possibly due to a moister climate. This in a way corroborates the observations on the mortality of the young scales during January, 1920. Mildew seems to form quickly in the scale mass when it becomes too moist. The preferred feeding place of the scale (the soft living tissues of the leaf bases and fiber strands) is found to be cool and slightly moist even on the hottest day. This possibly explains the mortality among the exposed scales under the hot, dry condition of summer.

CONDITION OF FRUIT STEMS AT DIFFERENT SEASONS

A study of the progress of the infestation on the leaf bases and fruit stems cut from heavily infested palms at different seasons of the year proved most interesting. It shows clearly how fruit stems of the current year become infested from the spring migration and also shows the development of the scale.

There are usually from 5 to 7 leaf bases between the tip of the crown and the fruit stems of the current year and five leaf bases between the fruit stems of each succeeding year. The leaf bases are arranged in a

spiral on the bole of the palm. The fruit stems come out from behind the leaf bases in a definite area around the bole each year.

August, 1919. Fruit stems of current year (1919) heavily infested with immature and mature (wine-red) stages. Leaf bases above (first two) show rapid decrease (almost clean) in amount of scale of similar development. Leaf bases (third, fourth, fifth) below fruit stem with increasing amounts of old brown and bronze-colored scales, but still some living immature and mature forms.

Fruit stems of 1918 dead and brown but covered at base with old dead scales. Leaf bases below with small margins of scales in all stages of development but decreasing in quantity.

November, 1919. Condition similar to that noted above but with increased amount of scale mass and more reaching brown stage.

April, 1920. (Migratory period beginning.)

Fruit stems of current year (1920) with only few (migratory forms) on outside of bases of spathe.

First leaf base above clean.

First leaf base below clean.

Second leaf base below with only a few very young scales in fiber.

Third leaf base below with young (migrating) forms settling.

Fourth leaf base below with young and a few mature (wine-colored) forms.

Fifth leaf base below heavily infested with wine-colored forms and with a few brown ones.

Fruit stem of 1919 heavily infested in all stages, mostly brown and bronze.

June 2, 1920. (Migratory period about over.)

Fruit stems of current year (1920) lightly infested from 6 to 18 inches from base with immature scales.

First leaf base above clean.

First leaf base below lightly infested with immature forms.

Second leaf base below lightly infested with immature and wine-colored forms.

Third leaf base below with medium infestation; some brown scales but mostly wine-colored and immature forms.

Fourth leaf base below heavily infested with brown and wine-colored forms, some immature at lower margin.

Fifth leaf base below heavily infested, all brown, mostly dead.

Fruit stems of 1919 heavily infested, all brown, dead.

NATURAL ENEMIES

Apparently there are few natural enemies of the red date-palm scale. Of greatest importance is the little beetle identified by Mr. E. A. Schwarz as *Laemophloeus* (*Cryptolestes*) *truncatus* Casey (?) found working throughout the year on the heavier infestations in the Mecca gardens. Occasionally definite galleries of destroyed scales were noted where these beetles were working. This beetle was also found feeding on the scales out on the fruit clusters in a commercial garden during the latter part of April.

In January, 1920, a number of orange-pink dipterous larvæ, possibly those of some species of Itonididae, were found under a leaf base in the midst of a mass of scale insects.

SUMMARY OF BIOLOGY

Following are the important points in the biological study in relation to control:

1. The most important means of distribution is through imported palms and offshoots from infested trees. These offshoots are invariably severely infested and carry the infestation through propagation to new plantings.

2. The main infestation on a mature palm is limited to the area extending 1½ to 3 feet below the crown, the crown tissues being free of scales. Lighter infestations continue on down the bole even to the ground.

3. From July to April practically all of the scales are concealed beneath the leaf and fiber bases and on the fruit stems of the current year.

4. The so-called spring migratory period of the scale is from April to June, and it is at this period that the new tissues and fruit stems become infested from the infested area of last year's fruit stems.

5. The majority of the exposed generations of the migratory periods out on the leaves are killed by the dry, hot weather following the migration, though some may persist in certain protected pinnæ and reproduce a limited number of scales.

6. The scale is found in all stages of development at all seasons of the year on the soft tissues of the leaf base and fiber band tissues. This indicates a continuous overlapping of generations. The duration of each generation is apparently from six to nine months.

CONTROL METHODS

Though the exposed red date-palm scale is readily killed by most contact insecticides its protected position under the leaf bases and tight fiber bands makes control by spray or fumigant most difficult. It is necessary to use a spray which will penetrate deeply between the fiber bands and leaf bases and to repeat the applications consistently in order to reach the maximum number of scales. As shown in the biological studies, it is important to check the migrations of the scale, thus preventing the new tissues and fruit stems from becoming infested.

The formula and spray calendar recommended by Mr. Drummond and Mr. Shamblin are as follows:

Liquor cresolis compositus, U. S. P. ..... 1 part
Distillate or kerosene ..... 4 parts.
Water ..... 50 parts.

If kerosene is used in making the stock solution, only the very best grade should be used, as oils of low specific gravity tend to retard good emulsion, in many cases injuring the offshoot or growing palm.

The spray is best applied by a power sprayer with a good agitator and under 200 to 225 pounds pressure, two leads of 30-foot spray hose being used. Six-foot to 12-foot spray rods with driving-spray nozzles set at

an angle of 45°, should be used. It is advisable to work from an elevated platform in spraying tall palms, rather than to use too long a rod.

Before attempting to spray, the palms should be properly pruned, surplus leaves being removed from the parent plant and the short method of pruning being used on the offshoots. Where possible, especially in the spring months, as many of the offshoots as are ready should be removed from the parent plant prior to spraying. On old palms the decayed leaf bases and infested superficial roots should be removed. If the dirt has been worked up around the base of the palm during cultivation this should be removed before spraying.

Too much importance can not be given to making a thorough application of the spray. Starting about a foot below the crown of the palm the solution should be so thoroughly applied as to soak all the fiber and completely fill the spaces between the leaf bases from this point to the ground. A slow, careful application made from at least two sides of the trunk is to be preferred to a hasty, incomplete spraying. The spraying of the old leaves to control the infestation in the leaf pinnæ may be limited to severely infested trees and especially immediately following the migratory periods. An average 10-year-old tree with a 6-foot trunk, properly pruned, will take from 15 to 20 gallons of spray.

The proper time to spray is determined and limited by the time the fruit crop is off, the migratory period of the scale, the blooming period, and the time the fruit begins to make sugar. On imported palms (barring seedlings) the fruit is generally all harvested by November 15, the blooming period is from the latter part of March on until May, the spring migration of the scale is from April to June, and the fruit begins to make sugar about August. This permits four sprayings per year, as follows:

First spray period	Jan. 1 to Feb. 15
Second spray period	Feb. 15 to Apr. 1
Third spray period	May 15 to June 30
Fourth spray period	July 1 to Aug. 15

On seedling palms the spray periods will of necessity be governed by the factors mentioned above and will also vary with different varieties. In most cases the spray periods will be of shorter duration, but even then not less than four sprayings should be given.

The treatment of infested offshoots as now practiced consists of thoroughly and completely dipping the cut offshoots in a bath of the foregoing solution. They should be submerged for 15 minutes, taken out and drained for 24 hours, and then submerged a second time for 15 minutes, making 30 minutes altogether in the solution. This solution at a strength of 1 to 50 will not injure the offshoot if left in it 24 hours. Offshoots should also be carefully inspected and sprayed during propagation planting.

PLATE 127

Showing nature of growth of leaves, fruit stems, and offshoots of date palm.

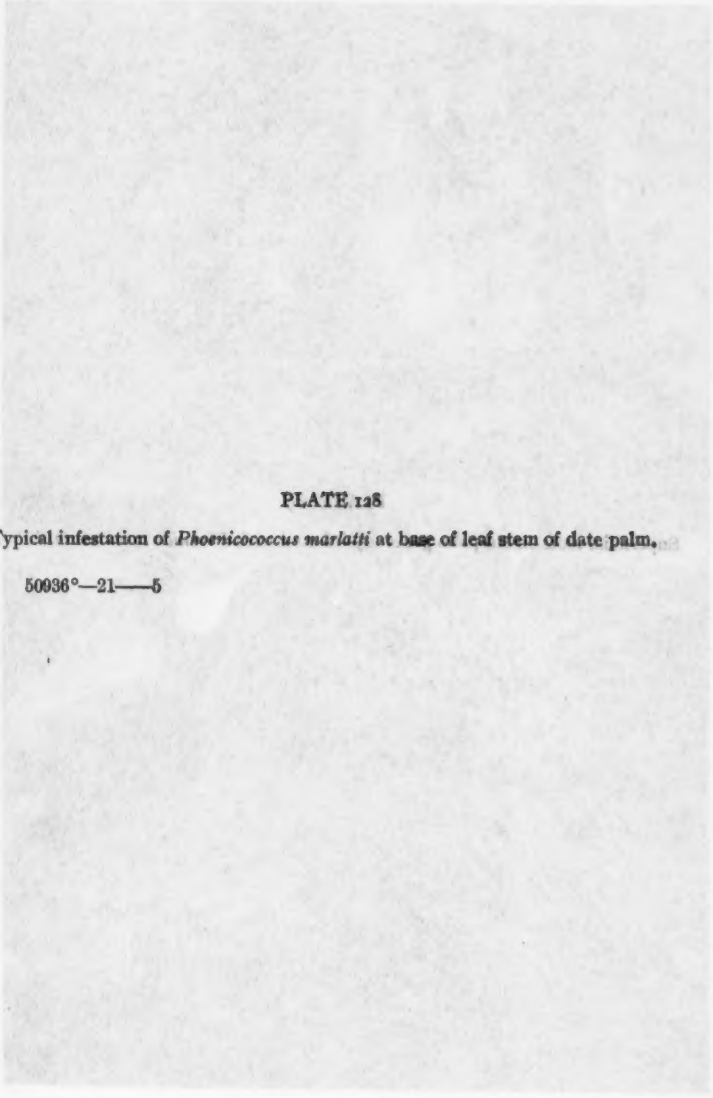

PLATE 128

Typical infestation of *Phoenicococcus marlatti* at base of leaf stem of date palm.

PLATE 129

Superficial roots on old palm generally infested with *Phoenicococcus marlatti.*

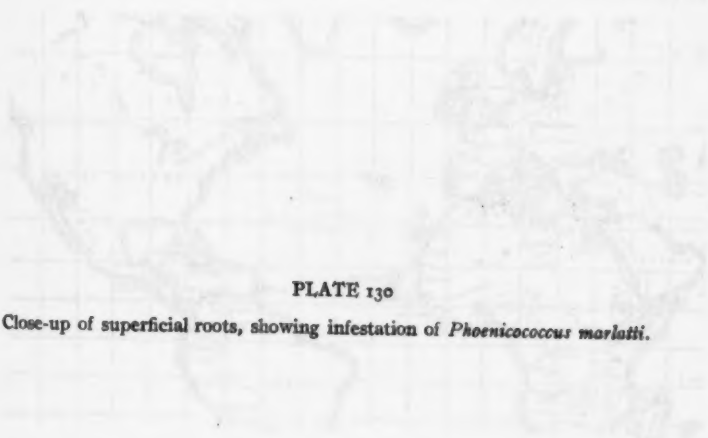

PLATE 130

Close-up of superficial roots, showing infestation of *Phoenicococcus marlatti*.

RED DATE-PALM SCALE, PHOENICOCOCCUS MARLATTI: A TECHNICAL DESCRIPTION

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The accumulation of information concerning the identity and distribution of *Phoenicococcus marlatti* Ckll., the biology of which is discussed by Borden (*26*),[2] has been largely due to the efforts of American entomologists and botanists interested either in the insect itself or in the introduction of the date palm into the United States. An examination of the specimens from which this species was described, preserved in the National Collection of Coccidae, shows that it was first collected by Dr.

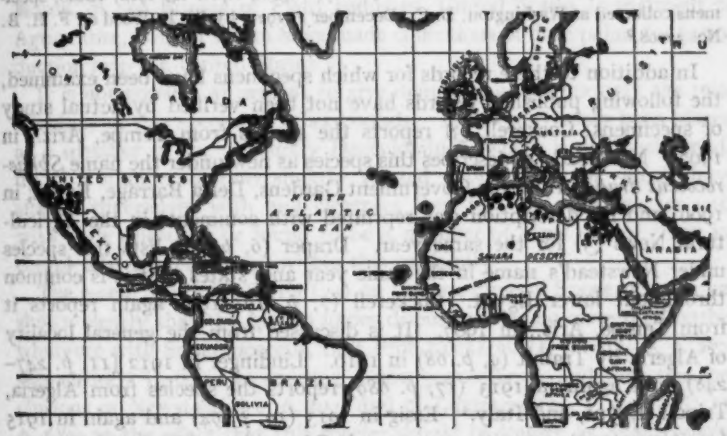

FIG. 1.—Present known distribution of the red date-palm scale (*Phoenicococcus marlatti*), the records based on specimens examined being indicated by large black dots and those based on published records of the occurrence of the species by small black dots.

C. L. Marlatt on August 7, 1890, on young date palms imported probably from Algeria and held at the time in the grounds of the Department of Agriculture in Washington. Subsequently the species has been recorded on many, if not all, of the importations of date palm plants from North Africa and Arabia. The National Collection of Coccidae at present includes specimens from the following definite localities, these being indicated by large round dots on the accompanying distribution map (fig. 1).

ASIA.—Bussorah, Arabian Gulf, specimens collected at Washington, D. C., June 9, 1905; Haifa, Palestine, specimens collected at Washington, D. C., January 30, 1912, by E. R. Sasscer on S. P. I. No. 32721 [3] (*15*).

[1] The drawings illustrating the structural characteristics of the insect described herein have been prepared by Emily Morrison, of the Bureau of Entomology, and the two photographic illustrations by E. R. Sasscer, of the Federal Horticultural Board.

[2] Reference is made by number (italic) to "Literature cited," p. 647–676.

[3] Foreign Seed and Plant Introduction accession number.

AFRICA.—Egypt: General locality, specimens collected at Tucson, Ariz., July, 1904, by R. H. Forbes; general locality North Africa, specimens collected at New York City, July, 1920, by H. B. Shaw on N. Y. F. H. B. No. 595[1]; Cairo, collected at Washington, D. C., February 9, 1912, by E. R. Sasscer; Alexandria, specimens collected at Washington, D. C., May 26, 1920, by H. Y. Gouldman on F. H. B. No. 31843.[2] Tunis: Souf Oases, Sahara Desert, specimens collected June 28, 1907, by W. T. Swingle on S. P. I. No. 21113[3] (*8*). Algeria: General locality, listed as "Algeria or Egypt" by the describer of the species, specimens collected at Washington, D. C., August 7, 1890, by C. L. Marlatt; Orleansville, place of collection not given, specimens collected by T. D. A. Cockerell, in 1899.

NORTH AMERICA.—California: Mecca, specimens collected by A. B. Staubenrauch in February, 1906; the same, specimens collected by A. J. Shamblin, August, 1914; the same, specimens collected at various times during 1918–1920 by A. D. Borden; Imperial Valley, specimens collected by C. J. Brand, April 14, 1908; Indio, specimens collected at Washington, D. C., December 2, 1920, by W. B. Wood on F. H. B. No. 32068.[2]

In addition to these records for which specimens have been examined, the following published records have not been verified by actual study of specimens: Cockerell (*3*) reports the species from Tempe, Ariz., in 1902. Newstead (*4*) describes this species as new under the name *Sphaerococcus draperi* from the Government Gardens, Delta Barrage, Egypt, in 1906, and his description was reprinted with comments in the Agricultural News (*5*) for the same year. Draper (*6*, *p. 12*) lists this species under Newstead's name in the same year and states that it is common throughout lower Egypt. Cockerell (*7*, *p. 191–192*) again reports it from Tempe, Ariz., in 1907. It is discussed from the general locality of Algeria by Trabut (*9*, *p. 68*) in 1910. Lindinger in 1912 (*11*, *p. 247–248*), and again in 1913 (*17*, *p. 689*) reports the species from Algeria, Tripoli, Egypt, and Italy.[4] Essig in 1913 (*19*, *p. 94*) and again in 1915 (*21*, *p. 123–124*) notes its occurrence in Riverside and Imperial Counties in California, as does Cook (*20*) in 1914. The Notice of Quarantine No. 6 of the Federal Horticultural Board of the United States Department of Agriculture (*13*) gives Riverside and Imperial Counties in California, Yuma, Maricopa, and Pinal Counties in Arizona, and Webb County in Texas as localities where the coccid now occurs in the United States. Forbes (*16*) in connection with a discussion of the control of date-palm scales gives Phoenix, Ariz., as a place where this species occurs; and, finally, Pierce (*22*, *p. 162*) in 1917 lists it from Algeria, Egypt, and Sahara as among the dangerous insects likely to be imported into the United States.[5] All of these published localities are indicated on the accompany-

[1] Federal Horticultural Board (Port of New York) accession number, unpublished.

[2] Federal Horticultural Board (Washington, D. C.) accession number, unpublished.

[3] Foreign Seed and Plant Introduction accession number.

[4] Lindinger (*14*) says (translation) "reported once from Italy." It has not been possible thus far to locate this report in any other coccid literature, and Leonardi (*22*) fails to include the species in his list of Italian Coccidae published in 1917.

[5] In an article appearing after the preparation of this description Buxton (*25*) notes the occurrence of this insect in Egypt and Algeria, its importation and establishment in California and Arizona, and his failure to locate it in Mesopotamia where he made an investigation of date-palm insects. He gives a fairly extended discussion of its habits and calls attention to a paper by Trabut (*12*) describing an Algerian disease of date palms caused by this scale insect.

ing distribution map (fig. 1) by small black dots. Besides these, Wilsie (*14*) and Popenoe (*18, p. 151*) discuss this insect without giving definite distribution records. In every case on record, the host of the insect has been some variety of the date palm, *Phoenix dactylifera* L., and all of the evidence now available indicates that it is probably confined to this host in nature.

The preceding definite records of the occurrence of this species indicate a very scattered distribution, but considering its habits and the methods employed by human agencies in distributing its host, it is highly probable that this scale insect actually occurs wherever the date palm is cultivated, as has been suggested by Dr. W. T. Swingle and Prof. S. C. Mason, of the Bureau of Plant Industry, United States Department of Agriculture, both of whom have made collections of date palms for introduction into the United States.

It is not possible at present to give definitely and accurately the relationships of this species to other coccids. It has previously been placed as a member of the subfamily Dactylopiinae, which is obviously made up of a number of only slightly related groups, few of which have shaped themselves with any clearness thus far; and this species will, with little question, be found to be a member of some one of these incompletely defined groups within this subfamily.

The recent study by Ferris (*24*) on the genotype of Sphaerococcus has shown clearly that the species under discussion has no close relationship whatever with Sphaerococcus, that Prof. Cockerell (*1, p. 262; 2, p. 277*) was entirely justified in erecting a new genus for it, and that the conclusions of Newstead (*10, p. 104*) and of Lindinger (*11, p. 247–248; 17, p. 689*) to the effect that this species should be known as *Sphaerococcus marlatti* rather than *Phoenicococcus marlatti* are incorrect.

The technical descriptions of the various stages of both sexes follow.

Phoenicococcus marlatti Ckll.

LARVA (Pl. 131, A–I).—Embryonic larva ready to emerge from female elongate oval, length about 0.27 mm., width about 0.13 mm.; antennæ 6-segmented, placed rather close together at anterior apex of body, front of head between bases straight, basal antennal segment much enlarged, 12 microns long by 25 microns wide, stout truncate-conical, the remaining segments much more slender, approximately cylindrical, the measurements of one antenna in microns as follows: II, 10.5; III, 7; IV, 7; V, 7; VI, 18; legs small, the basal portion stout and the tarsus somewhat swollen, tarsal claw with denticle, the approximate measurements of a middle leg in microns as follows: Coxa, 11; trochanter, 4; femur, 25; tibia, 11; tarsus, 11; claw, 7; digitule about 10.5; beak apparently 1-segmented; with two pairs of thoracic spiracles, these small, each with a relatively long, flat, chitinized bar attached, and each with a single quinquelocular pore just anterior to the opening; derm with seven pairs of long tubular ducts, each terminating in a bilocular 8-shaped pore at its inner end, opening along the body margin, these two pairs on the head, one pair on the mesothorax, one on the metathorax, and one pair each on the first, fourth, and terminal abdominal segments; dorsally with three pairs of similar, but smaller ducts, one on each side about half way

between middle line and body margin, one pair on posterior half of mesothorax, one on anterior half of metathorax, one on first abdominal segment, no types of pores other than these two observed; derm bearing only small, slender setæ, these in longitudinal rows, with a submedian and a submarginal both dorsally and ventrally, and a marginal row, this last having the smallest setæ, with one seta of each row to each segment, at least on the abdomen; a few of the setæ near the antennæ much larger; apex of body terminating with two pairs of very large setæ, relative to those on the body, the outer pair about 53 microns long, the inner about 125 microns; without traces of anal lobes; anal ring small, simple, flattened and extended below, with a more heavily chitinized inner margin, placed apically or a little ventrally, bearing two small setæ above, two near the lower edge of the opening, and two, larger, at the lower edge of the chitinization.

SECOND-STAGE FEMALE (Pl. 132, A–D).—Shape variable, according to stage of development, shortly after first molt somewhat ovate, broadest a little anterior to middle, tapering posteriorly, rounded anteriorly, length at this time 0.32 mm., width about 0.185 mm., later enlarging and approaching more nearly the broad oval form found in the typical adult; derm clearing completely on being treated with caustic potash, showing many small, angularly pointed papillæ, these much less conspicuous in the older swollen specimens, not in distinct close clusters, but scattered along the body margin and in the submarginal area beneath, largest anterior to the antennæ; antennæ placed ventrally near anterior apex of body, nearer to margin than to each other, very much reduced, apparently 1-segmented, the apex slightly invaginated and bearing five small, stout setæ; without traces of the eye spots or legs in mounted specimens; framework of mouthparts large and conspicuous, beak very short and stout, apparently 1-segmented, but this not definitely determinable; spiracles with a circular opening at the outer end of an elongate chitinous bar having a somewhat expanded inner end, and with a loose cluster of 4 to 7 relatively large, quinquelocular disk pores around the opening, the anterior spiracles usually with one or two more of these than the posterior pair; derm along the margin, and in the submarginal region ventrally, with a number of the long tubular ducts already described for the larva, these scattered rather uniformly, varying somewhat in size and much less numerous than the derm papillæ; body dorsally, at the margin, and ventrally with a few longitudinal rows of short and stout, acute setæ; anal ring placed ventrally near the posterior apex of the body, small, simple, the opening somewhat kidney-shaped, retaining a single pair of small setæ, these morphologically at the lower angles of the ring.

ADULT FEMALE (Pl. 133, A, D–M; Pl. 134).—The living specimen, lying on the surface of the host tissue, more or less surrounded by a rim of dense waxy secretion, this ordinarily white, but often discolored through the incorporation of particles of foreign matter; body varying considerably in shape, stout oval, often somewhat broader behind, not strongly convex, the dorsal surface flattened, ventrally excavated along the middle line, presumably to furnish a space for the emergence of the larvæ; body color reddish, except for the central area, this appearing pale greenish yellow; dorsally faintly shining; with some fine, scattered, curved, glassy threads of secretion dorsally, particularly near the margin; color ventrally similar to that of dorsum, with the large groove already mentioned more or less filled with a loose, fluffy secretion of glassy threads similar to those occurring dorsally, but much longer, much more abundant, and curled.

Adult female morphologically stout and broad oval, often nearly circular, length just after molting about 0.5 mm., width about 0.37 mm., maximum length of specimens examined 1.4 mm., width 1.1 mm.; derm just after molting clearing completely on being treated with caustic potash, the derm around the margin showing a broad band of closely crowded, elongate papillæ, each with serrate or denticulate apex, and dorsally, laterally and ventrally longitudinal rows of small, stout setæ; fully matured

adult, as indicated by the measurements given, considerably larger and broader, derm remaining more or less chitinized and yellow brown on being treated with caustic potash, the extent of this apparently depending on the age of the specimens at the time of their separation from the living host; antennæ placed ventrally near the body margin, separated from each other by a distance slightly greater than the width of the framework of the mouthparts, very small, apparently 1-segmented, and of peculiar construction, the apex invaginated for fully half its length, and bearing five short spines at the bottom of the invagination, these protruding little, if any, beyond the mouth of the invagination; legs entirely wanting; framework of mouthparts large, but relatively less conspicuous than in preceding stage; beak small and stout, conical, segmentation not definitely determinable; spiracles with an elongate bar, expanded at inner end, with a loose cluster of from 12 to 15 quinquelocular disk pores around the opening, and with a varying number of similar pores scattered beyond each spiracle towards the margin, these particularly noticeable opposite the anterior pair; derm with the papillæ remaining closely crowded at posterior and anterior apices of body, but much more scattered along lateral margins where the principal expansion due to growth evidently occurs; derm with only one other type of pore in addition to the spiracular pores, this a long tubular duct with bilocular inner end, similar to those of larva and second stage, but much more abundant, varying somewhat in size, a few scattered dorsally, but mostly occurring along the margin and ventrally, never in clusters, although more numerous in certain areas, as throughout the ventral groove region, than in others; derm with an occasional small seta, these most numerous in the midventral abdominal area; anal ring placed ventrally near the posterior apex of the body, small, simple, heavily chitinized, retaining the two lower setæ only as in preceding stage.

MALE LARVA.—Apparently indistinguishable from that of female.

MALE SECOND-STAGE LARVA (Pl. 131, J–M).—Rather elongate ovoid, broadest before the middle, length about 0.32 mm., width about 0.18 mm., probably flattened; antennæ placed near anterior apex of body, separated by the width of the framework of the mouthparts, small and short tubular, apparently 1-segmented, more or less invaginated at apex; legs entirely wanting; framework of mouthparts very large and conspicuous, beak small, short conical, segmentation not determinable; spiracles small, with a heavy chitinous bar somewhat expanded at inner end, each accompanied by a single, normally quinquelocular disk pore; derm with some of the tubular ducts of other stages along the margin of the body and in the submarginal area ventrally; derm, at least on the abdomen, with eight longitudinal rows of small setæ, one seta of each row to a segment, two rows dorsal, two marginal, and four ventral; derm with a few small papillæ around the posterior apex of the abdomen, these quite inconspicuous; anal ring small, simple, kidney-shaped, retaining the lower two small setæ, and showing a slight chitinized connection with the still lower pair of large setæ, but these well removed from the ring proper.

MALE PUPA (Pl. 131, N; 132, E).—Elongate oval, length about 0.36 mm., width about 0.16 to 0.18 mm.; antennæ and legs large and conspicuous, showing distinct segmentation in stained specimens, all these long conical, tapering from base to apex, the antennæ placed at the anterior apex of the body and protruding laterad and a little backwards, showing five distinct segments; anterior legs pointing cephalad, parallel and somewhat overlapping the antennæ, intermediate legs directed backwards and outwards, posterior legs extending backwards, nearly parallel and nearly reaching the apex of the abdomen, all the legs showing five distinct segments in stained specimens; mouthparts wanting, the derm with a large, roughly circular, chitinized area at the point where these occur in other stages; spiracles small and stout, without quinquelocular or other pores; derm, so far as can be noted, entirely without specialized secreting pores or ducts; with eight rows of tiny circles, these

possibly normally spine-bearing, two dorsally, two ventrally, two close to margin dorsally, and two submarginal ventrally; body terminating in a heavy, short, cylindrical protuberance having an irregular transverse opening in its center.

ADULT MALE (Pl. 133, B, C, N-R).—Elongate, slender, sides nearly parallel, rounded anteriorly and tapering to a point posteriorly, dimensions from specimens mounted alive in balsam, length 0.42 mm., width 0.14 mm.; antennæ stout, placed close together at anterior apex of body, the two bases separated by perhaps half the width of the basal segment, this much broader than long, the second segment stout, somewhat larger apically, the third segment with a slender stalklike base, this with several grooves and ridges running around it, but the apical three-fifths strongly expanded, fourth segment also triangular, but stouter at base than third, remainder of antenna, in specimens mounted in balsam while alive, appearing as three distinct but closely united segments, this appearance much less conspicuous in specimens treated with caustic potash and stained, but still indicated, the antennæ, therefore, probably 7-segmented with the five terminal ones forming an elongate club terminating in a stout button appearing more or less distinctly set off by a joint between it and the club proper, as if it might be still another segment; so far as can be determined, with two long, slender setæ on II, III, and IV, with four similar at the apex of the club, and with one stout spine on V, one, longer, on VI, and two, still larger, on VII; measurements of one antenna in microns as follows: I, 14.2; II, 17.5; III, 14; IV, 10.7; V, 10.4; VI, 7.4; VII, 14.3 (total of club apex 32.1); legs short and stout, tibia and tarsus of each bearing one or more large, round, apparently sensory pores; lengths in microns of outer portion of a middle leg: Femur, 32; tibia, 18; tarsus, 14.3; claw, 10.7; digitules about 14; claw with denticle, digitules slender, slightly knobbed at tips; eyes simple, only one pair present, these as spots on margin a little posterior to the antennæ; mouthparts entirely wanting; wings entirely wanting; spiracles small, the bar stout, without any accessory pores; derm, so far as can be determined, entirely without specialized secreting pores, bearing, however, a number of tiny chitinized circles, these occurring singly, but probably in definite arrangement, normally seta-bearing; penis elongate, slender, apparently cylindrical, the surface roughened; sheath elongate, tapering, pointed, length about 30 microns, width close to base about 7.5 microns, suddenly expanding at base to about 14 microns.

In preparation of the preceding description specimens of one or more stages of the species from each of the lots of material now in the National Collection of Coccidae have been examined.

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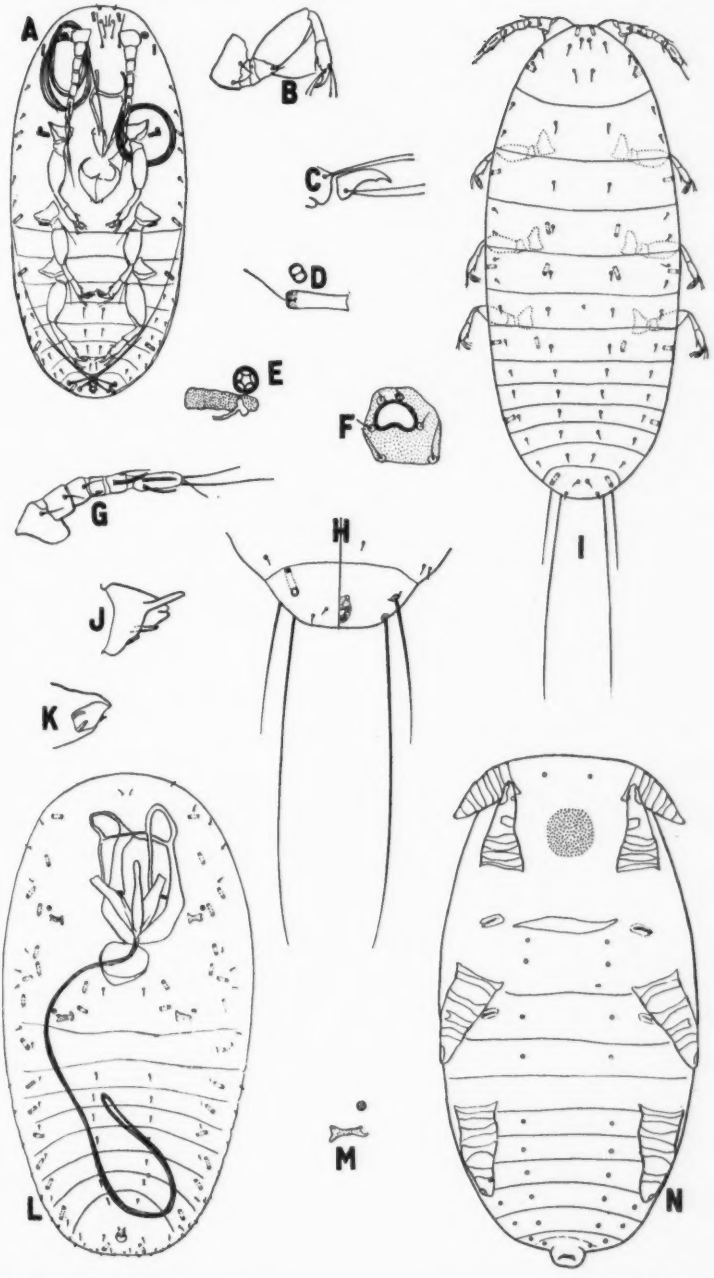
PLATE 131

Phoenicococcus marlatti:

A.—Embryonic larva. × 220.
B.—Larval leg. × 450.
C.—Larval claw. × 1,350.
D.—Larval tubular duct. × 1,350.
E.—Larval spiracle. × 1,350.
F.—Larval anal ring. × 1,350.
G.—Larval antenna. × 450.
H.—Larva, apex of abdomen. × 450.
I. Larva, outline from above. × 220.
J.—Male second-stage larva, antenna. × 1,350.
K.—Same, another antenna. × 1,350.
L.—Male second-stage larva, outline of body from beneath. × 220.
M.—Male second-stage larva, spiracle. × 450.
N.—Male pupal skin. × 220.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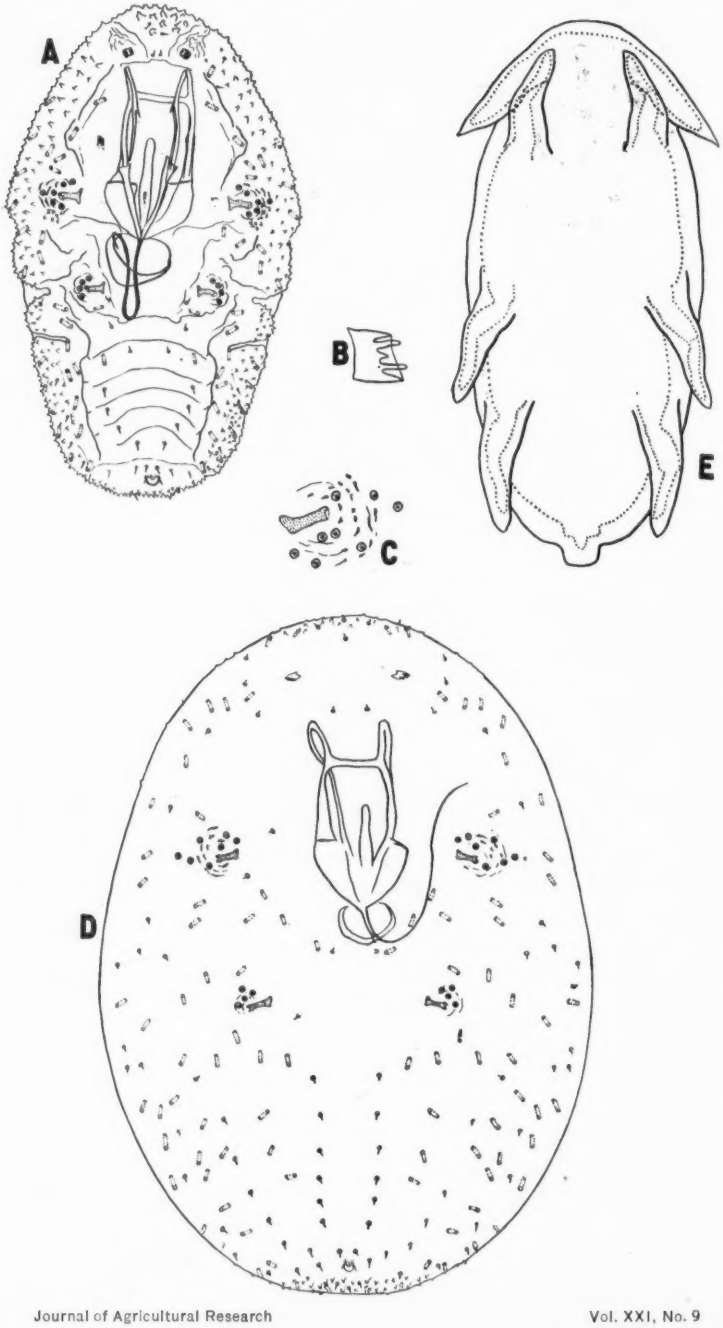
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PLATE 132

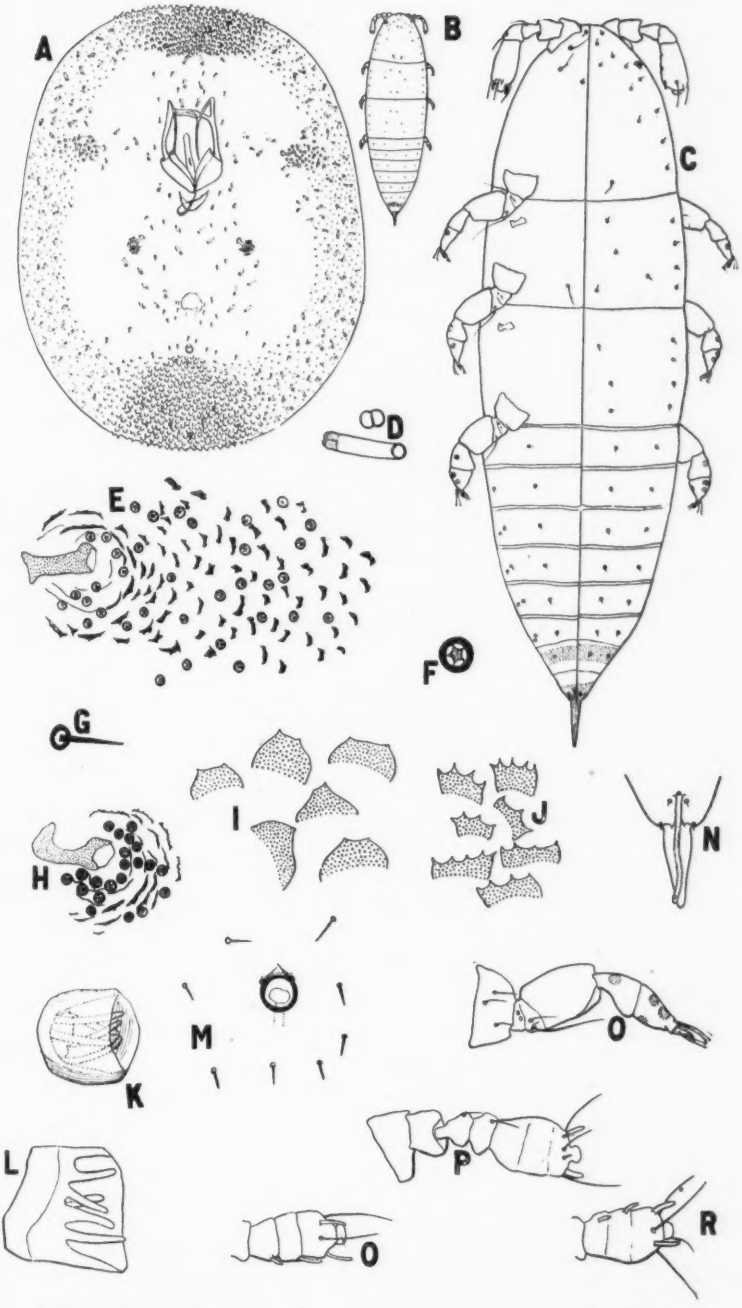
Phoenicococcus marlatti:

A.—Female, second-stage larva, just after molt. × 220.
B.—Same, antenna, optical section. × 450.
C.—Same, anterior spiracle. × 450.
D.—Same, outline of body after expansion to normal size. × 220.
E.—Male pupa, outline, showing half-formed adult male inclosed. × 220.

PLATE 133

Phoenicococcus marlatti:

A.—Adult female, outline from beneath. × 70.
B.—Adult male, outline from above for comparison with female. × 70.
C.—Adult male, outline from above. × 220.
D.—Adult female, tubular duct. × 1,350.
E.—Adult female, anterior spiracle. × 450.
F.—Adult female, spiracular pore. × 1,350.
G.—Adult female, derm seta. × 1,350.
H.—Adult female, posterior spiracle. × 450.
I.—Adult female, derm papillæ from old, distended body. × 1,350.
J.—Adult female, papillæ from body before distension. × 1,350.
K.—Adult female, antenna. × 1,350.
L.—Same, crushed and flattened, showing the setæ. × 1,350.
M.—Adult female, anal ring and surrounding area. × 450.
N.—Adult male, penis and sheath. × 450.
O.—Adult male, leg. × 450.
P.—Adult male, antenna from cleared and stained specimen. × 450.
Q.—Tip of same from specimen mounted alive, showing apparently distinct segmentation. × 450.
R.—Same, tip of antenna figured at P, showing opposite surface. × 450.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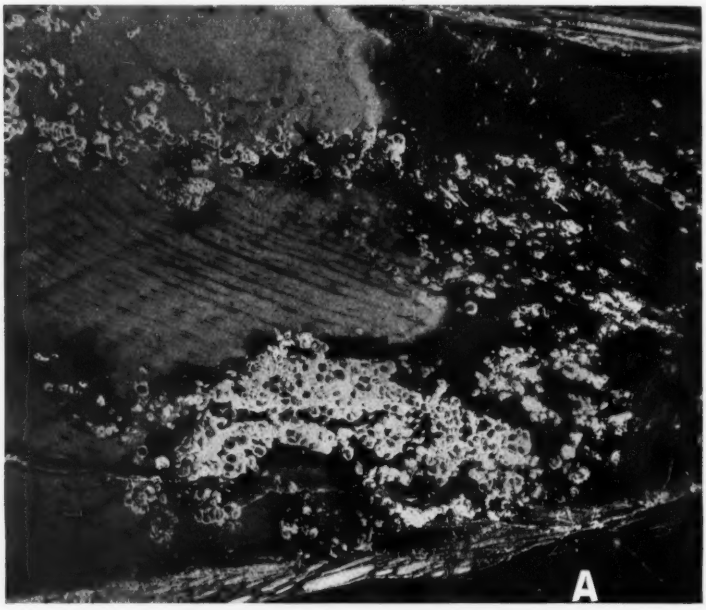
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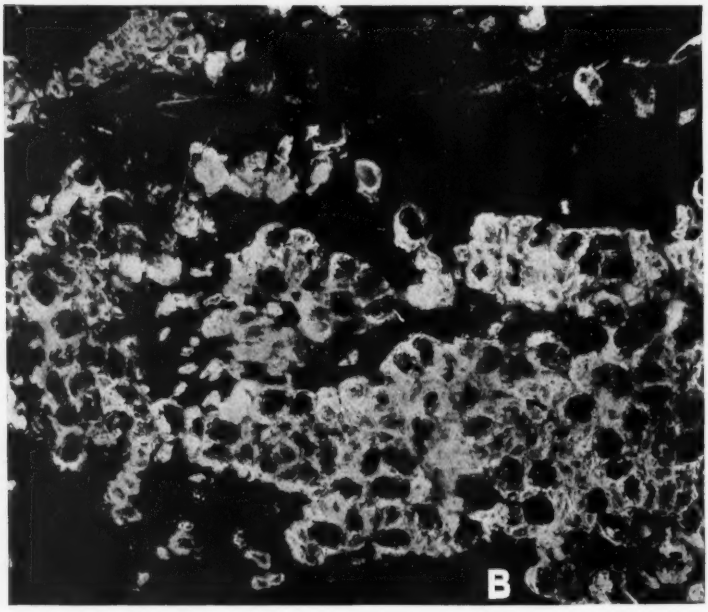
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PLATE 134

Phoenicococcus marlatti:

A.—Mass of females inside base of palm leaf.
B.—Portion of the same mass still more enlarged.

50936°—21——6

SOME OBSERVATIONS REGARDING EOSINOPHILES

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During the past few years our knowledge of the relationship of a parasitic infestation and an eosinophilia has been undergoing considerable revision. Not so very long ago the presence of an unusually large number of eosinophiles was considered almost diagnostic of a severe infestation with some form of external or internal parasites. Also an absence of an eosinophilia presented very strong evidence that the animal was almost if not entirely free from parasitism.

The observations reported here are based on a study of 40 cases. From 1 to more than 20 blood examinations have been made on the individual cases, some of which extended over a period of about 18 months. In all the cases the animals were carefully autopsied and a close search for internal parasites was made. None of the animals were harboring external parasites.

The usual precautions for drawing the blood and making the examinations were observed. The place chosen for procuring the blood was the under surface of the tail. In case this failed, the skin was shaved at the shoulder and the incision made there. As a rule, Tallquist's hemoglobin scale was used, although Gower's method was used in some instances. Both red and white cells were counted in the same preparation, a double-ruled Neubauer counting chamber being used in most cases. For the differential count Giemsa's stain was ordinarily used, but Wright's and Jenner's were sometimes used. Five hundred cells were usually counted, although sometimes 1,000 were counted.

The writer is indebted to Dr. Edward Records for checking some of the differential counts. While the two counts did not absolutely check, they were very close. It is not to be expected that even two counts by the same person on the same slide would be identical.

[1] Resigned June 1, 1920.

TABLE I.—*Blood counts of horses in apparent good health*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Apr. 30, 1918	70	6,572,000	7,130	38.4	51.0	4.0	5.4	1.2	0
	May 6, 1918	77	7,348,000	6,920	40.8	44.6	8.4	5.6	.6	0
	May 18, 1918	90	7,420,000	6,300	41.6	54.2	4.0	.2	0	0
	May 28, 1918	95	8,000,000	9,360	48.2	46.6	3.4	1.6	.2	0
	June 4, 1918	97	8,800,000	8,000	34.6	50.2	10.2	3.0	2.0	0
	June 14, 1918	101	7,960,000	7,260	34.0	51.4	10.0	3.4	1.2	0
	June 19, 1918	103	9,126,000	9,760	30.0	63.8	4.4	1.4	.4	0
673	June 25, 1918	97	7,704,000	5,940	29.4	53.6	6.8	9.2	1.2	0
	July 9, 1918	105	9,010,000	6,660	33.2	51.0	7.2	8.2	.4	0
	July 23, 1918	80	7,642,000	5,850	26.4	56.2	8.0	9.4	0	0
	Aug. 6, 1918	100	7,888,000	7,740	24.4	63.4	9.2	3.0	0	0
	Aug. 26, 1918	94	6,432,000	9,777	33.2	54.4	8.0	4.2	.2	0
	Oct. 8, 1918	100	7,880,000	8,349	21.4	65.2	11.4	2.0	0	0
	Oct. 27, 1918	100	6,888,000	9,861	38.8	39.6	14.6	7.0	0	0
	Dec. 22, 1918	75	7,296,000	7,633	37.4	55.2	6.2	1.2	0	0
634	Oct. 8, 1918	92	7,880,000	9,873	22.6	68.2	9.2	0	0	0
15	May 29, 1919	100	7,964,000	8,291	33.2	60.2	4.2	2.4	0	0
	Dec. 16, 1918	90	7,842,000	7,383	37.6	54.2	5.6	2.6	0	0
	Jan. 3, 1919	100	8,306,000	9,471	30.2	60.0	5.8	4.0	0	0
	Mar. 1, 1919	90	7,684,000	9,021	31.8	57.6	10.2	2.4	0	0
2	Apr. 8, 1919	90	7,964,000	9,769	41.2	52.4	4.4	2.0	0	0
	Apr. 25, 1919	90	7,964,000	7,783	28.2	61.4	7.4	3.0	0	0
	May 16, 1919	100	8,264,000	5,396	30.0	63.2	4.2	2.6	0	0
	May 27, 1919	100	8,032,000	5,387	31.3	57.4	6.6	4.2	0	0
	Sept. 3, 1918	98	6,912,000	8,888	40.2	54.2	3.0	2.6	0	0
	Oct. 10, 1918	90	6,834,000	9,873	37.2	53.0	13.0	6.8	0	0
754	Nov. 2, 1918	88	7,432,000	5,961	34.6	55.4	8.4	1.6	0	0
	Dec. 22, 1918	100	8,264,000	9,666	46.2	49.0	3.6	1.0	.2	0
702	Oct. 27, 1918	85	7,480,000	8,677	63.2	27.4	8.2	1.2	0	0
	May 20, 1918	95	8,288,000	9,400	29.8	63.2	4.4	2.0	.6	0
	May 28, 1918	100	7,216,000	7,920	37.6	53.2	2.6	6.4	.2	0
	June 3, 1918	106	9,260,000	8,330	36.6	50.4	6.2	5.8	1.0	0
	June 12, 1918	105	10,624,000	8,280	29.2	60.2	6.0	3.6	1.0	0
501	June 17, 1918	104	10,496,000	8,000	31.2	50.2	12.2	6.2	.2	0
	June 25, 1918	89	6,688,000	5,580	31.2	54.4	10.2	3.8	.4	0
	July 9, 1918	93	8,340,000	9,360	42.8	50.4	2.2	4.4	.2	0
	July 6, 1918	100	6,384,000	7,200	27.3	56.2	10.2	6.3	0	0
	July 26, 1918	100	7,634,000	10,326	35.4	42.2	12.0	10.4	0	0
	Oct. 27, 1918	90	6,408,000	6,339	36.2	47.8	13.2	2.8	0	0
	Dec. 16, 1918	80	6,848,000	9,333	38.8	46.2	6.2	8.6	.2	0
	Dec. 22, 1918	90	7,362,000	8,431	40.0	37.0	7.8	15.2	0	0
	Jan. 2, 1919	80	7,642,000	4,420	34.2	38.2	7.4	20.2	0	0
	Jan. 28, 1919	100	6,973,000	8,821	24.6	53.4	8.0	14.0	0	0
8	Mar. 1, 1919	90	7,552,000	8,361	23.4	57.8	3.2	15.6	0	0
	Mar. 25, 1919	90	7,244,000	5,391	37.2	40.4	6.4	16.0	0	0
	Apr. 19, 1919	90	7,648,000	7,291	26.3	52.6	3.2	17.4	0	0
	Apr. 25, 1919	80	7,288,000	11,291	26.4	52.8	3.6	17.2	0	0
	May 26, 1919	90	7,648,000	9,361	23.2	56.6	3.8	16.4	0	0
	June 2, 1919	90	7,932,000	8,341	32.2	61.4	5.0	1.4	0	0
9	Apr. 8, 1919	90	7,860,000	4,381	37.8	54.2	3.6	4.4	0	0
	July 5, 1919	100	7,694,000	5,363	30.2	62.4	6.0	1.4	0	0
	Mar 18, 1920	100	8,064,000	7,010			No count made.			
	Sept. 18, 1917	105.6	11,972,000	5,330	46.2	36.2	10.2	7.0	.4	0
	Sept. 25, 1917	95	8,584,000	10,666	47.8	37.0	7.4	7.6	.2	0
570	Sept. 28, 1917	95	8,032,000	7,333	38.0	50.4	6.4	4.6	.2	0
	Oct. 6, 1917	60	5,122,400	1,780	29.8	67.6	2.2	.4	0	0
	Oct. 11, 1917	70	5,824,000	7,555	57.2	26.6	13.2	3.0	0	0
	Oct. 24, 1917	.....	5,072,200	4,666	46.8	46.8	6.0	.4	0	0

TABLE I.—*Blood counts of horses in apparent good health*—Continued

Tag No.	Date.	Homoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Nov. 27, 1917	65	6,228,000	5,620	45.8	47.4	6.6	.2	0	0
	Apr. 30, 1918	90	6,912,000	7,480	44.3	37.8	10.1	7.1	.7	0
	May 6, 1918	90	8,080,000	9,430	50.4	39.0	7.6	3.0	0	0
	May 10, 1918	80	10,420,000	9,760	36.0	51.0	6.8	4.6	1.2	0
	May 16, 1918	80	8,040,000	9,460	37.2	48.2	5.2	6.6	.8	0
	May 24, 1918	80	7,784,000	8,000	28.0	50.2	10.0	11.2	.6	0
	June 3, 1918	100	8,840,000	7,420	26.4	64.2	5.8	3.6	0	0
570	June 12, 1918	100	7,482,000	7,020	38.4	51.8	7.0	2.6	.2	0
	June 18, 1918	100	8,342,000	6,430	33.4	58.2	5.6	2.4	.4	0
	June 24, 1918	101	9,280,000	4,500	28.0	61.6	6.8	3.6	0	0
	July 8, 1918	95	8,684,000	9,360	36.4	58.2	4.4	1.0	0	0
	July 23, 1918	100	8,560,000	5,588	23.2	72.4	2.4	2.0	0	0
	Sept. 3, 1918	90	7,704,000	8,000	33.4	60.2	4.2	2.0	.2	0
	Oct. 10, 1918	100	8,324,000	5,386	38.6	48.4	10.0	2.6	.4	0
	Oct. 27, 1918	100	7,264,000	11,133	32.0	59.2	5.6	3.2	0	0
7	Dec. 16, 1918	80	7,208,000	6,339	41.4	48.6	7.2	2.4	.4	0
	Feb. 22, 1919	90	6,974,000	8,360	32.6	57.0	8.2	2.2	0	0
13	Mar. 8, 1919	90	7,382,000	5,981	33.4	61.2	2.8	2.6	0	0
	Mar. 25, 1919	80	7,164,000	5,089	22.0	67.2	7.2	3.6	0	0
633	May 2, 1918	70	8,490,000	11,400	33.6	54.6	4.4	6.4	1.0	0
1	Nov. 14, 1918	90	7,232,000	8,169	26.6	55.2	5.2	13.0	0	0
	Average..	90.4	7,783,300	7,693	34.8	52.9	6.8	5.3	.2	0

No. 673.—Aged, healthy gelding in good physical condition.

At autopsy, December 30, 1918, there were found 1 *Gastrophilus* spp., 3 Setaria in the abdominal cavity and a few Strongylus and Cylicostomes in the cecum and colon.

No. 634.—Normal gelding 12 years old.

At autopsy, October 26, 1918, not a parasite of any kind was found.

No. 15.—Normal 15-year-old mare.

At autopsy, May 29, 1919, about 30 *Gastrophilus* spp. were in the pylorus. There was a moderate infestation with Strongylus and Cylicostomes in the cecum and colon. Two *Setaria equina* were found free in the abdominal cavity.

No. 2.—Normal aged gelding.

At autopsy, May 27, 1919, about 350 *Gastrophilus* spp. were found in the pylorus; 1 *Ascaris equorum* was in the duodenum; there was a moderate infestation of Strongylus and Cylicostomes in the cecum and colon; and 25 Setaria were found in the abdominal cavity. There was a severe aneurism of the anterior mesenteric artery which contained live embryos.

No. 754.—Normal 15-year-old mare.

At autopsy, December 30, 1918, there were found 50 *Gastrophilus* spp. in the pylorus and moderately severe infestation with Strongylus and Cylicostomes in the cecum and colon.

No. 702.—Healthy 10-year-old gelding.

At autopsy, December 19, 1918, there were found 10 *Gastrophilus* spp. in the pylorus, about 75 Strongylus in the cecum and colon, and 1 Strongylus and 4 *Setaria equina* in the abdominal cavity.

No. 501.—Aged, healthy mare.

At autopsy, December 16, 1918, there was found a moderate infestation of Strongylus and Cylicostomes in the cecum and colon.

No. 8.—Aged, healthy gelding.

At autopsy on May 26, 1918, there were found about 150 *Gastrophilus* spp. in the stomach and pylorus, a moderate infestation of Strongylus and Cylicostomes, and a small aneurism of the anterior mesenteric artery containing live embryos.

No. 9.—Healthy 12-year-old mare.

At autopsy on March 18, 1920, there were found about 150 *Gastrophilus* spp., a severe infestation with Strongylus and Cylicostomes, and a small worm-free aneurism of the anterior mesenteric artery.

No. 570.—Five-year-old gelding in excellent condition.

At autopsy, December 16, 1918, there were found 6 *Gastrophilus* spp. and a most severe infestation of Strongylus and Cylicostomes. The animal showed no symptoms of the infestation. About two months before autopsy he was corralled with wormy colts, and it seems likely that the infestation came at that time from them.

No. 7.—Aged, healthy mare.

At autopsy, December 20, 1918, there were found a few *Gastrophilus* spp., 2 Setaria in the abdominal cavity, small number of Strongylus and Cylicostomes in the cecum and colon, and a few Oxyuris in the rectum.

No. 13.—Aged, healthy animal.

At autopsy, April 5, 1919, there were found 150 *Gastrophilus* spp., 1 Setaria, a few Strongylus and Cylicostomes in the cecum and colon, and a very large aneurism in the anterior mesenteric artery containing many live embryos.

No. 633.—Young, healthy gelding.

At autopsy, July 8, 1918, there were found a few *Gastrophilus* spp. and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 1.—Aged, healthy mare.

At autopsy, December 12, 1919, there was found a severe infestation of the cecum and colon with Strongylus and Cylicostomes.

DISCUSSION

The percentage of eosinophiles in the peripheral blood of healthy horses varies greatly even when the examinations are made at frequent intervals. In studying Table I, one can readily see that if certain individual examinations were picked out, exceedingly varying results might be obtained. This fact possibly accounts for many of the different

conclusions arrived at by many writers regarding the percentages of eosinophiles in the blood of normal horses or horses suffering from various diseases. Another fact that is apparently often if not always overlooked is the resistance of the animal. Weinberg and Seguin (*10*) [1] have pointed out that the eosinophiles possess specific attraction against certain toxic substances, especially those of parasitic origin. It would seem from this that if the resisting power of the animal were high the eosinophiles would be plentiful in parasitic diseases. If the resistance were low the eosinophiles would be few.

In general it can be said that in the various species of animals the physiological functions of the leucocytes are essentially the same. If this be so, cattle must be very severely infested with parasites or else their resistance is high. Dimmock and Thompson (*4*) have shown that the average percentage of eosinophiles in the blood of normal cattle is 13.15. Whatever the cause may be, it certainly presents a very interesting and important problem for further research. According to the tables given by Burnett (*2*) the percentages of eosinophiles are about the same for all animals excepting cattle.

One thing that adds difficulty to the study of the blood in apparently normal animals is that nearly all are harboring more or less large numbers of parasites of various genera. Two apparently normal animals observed have presented a very high percentage of eosinophiles. No. 8 for nine examinations over a period of six months averaged 17.2 per cent of eosinophiles. On autopsy, this animal showed a relatively small number of internal parasites. No. 1 for one examination showed 13 per cent, and on autopsy there were found large numbers of internal parasites.

As these were the only two animals that showed unusually large numbers of eosinophiles, very little more can be said about their presence as an aid to the diagnosis of internal parasites. One examination can be picked from some of the other cases that shows nearly as high a percentage as did No. 1. Again, it can be said that results will be conclusive only as the observations are made over a period of time on the same animal. Then also, one must consider that for some cause the animals might become more severely infested with parasites, or else they might get rid of them. There is apparently some seasonal variation, especially in regard to the intestinal worms, more being present in the fall and winter than in the spring and summer. This also should be considered.

[1] Reference is made by number (italic) to "Literature cited," p. 688.

TABLE II.—*Blood counts of horses in poor condition due to internal parasites*

Tag No.	Date.	Homoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	1918.									
A	Sept. 11	70	3,888,000	6,333	32.6	57.8	5.4	3.4	0.8	0
	Oct. 15	62	5,120,000	7,833	31.0	62.6	6.0	.4	0	0
B	Oct. 19	63	5,880,000	9,766	33.4	58.2	4.6	3.6	.2	0
	Oct. 29	60	5,608,000	7,291	31.4	58.4	8.4	1.6	.2	0
C	Sept. 11	80	6,424,000	7,333	28.4	60.2	7.2	4.2	0	0
	Oct. 1	.....	5,352,000	7,555	32.4	57.2	9.0	1.4	0	0
E	Sept. 11	60	8,640,000	8,342	30.6	41.2	27.0	1.2	0	0
	Oct. 23	90	7,232,000	6,773	28.8	62.4	5.8	3.0	0	0
	Oct. 29	70	6,980,000	9,639	42.6	33.0	18.6	5.8	0	0
F	Sept. 11	80	6,432,000	7,963	43.6	39.4	15.2	1.6	.2	0
	Oct. 19	60	4,400,000	6,839	36.2	70.2	3.6	0	0	0
	Oct. 21	63	5,568,000	13,867	30.8	58.6	7.0	3.6	0	0
G	Sept. 12	70	4,524,000	8,327	32.8	52.4	12.0	2.8	0	0
	Oct. 18	60	4,634,000	4,837	24.4	67.2	7.8	.6	0	0
H	Sept. 12	80	5,728,000	11,149	26.4	42.0	28.6	3.0	0	0
	Oct. 19	53	4,288,000	7,983	39.8	54.8	3.6	1.8	0	0
D	Sept. 11	70	6,080,000	12,642	33.8	52.2	10.6	3.4	0	0
	Oct. 23	76	6,832,000	7,637	24.6	58.0	16.0	1.2	.2	0
	Oct. 29	80	7,008,000	6,890	45.2	32.6	22.0	.2	0	0
	Average.	69.2	5,822,000	8,363	33.1	53.6	11.4	2.2	.08	0

A.—Three-year-old gelding with Strongylosis.

At autopsy October 15, 1918, there were found about 150 *Gastrophilus* spp. in the pylorus, a serious infestation of Strongylus and Cylicostomes in the cecum and colon, and a small aneurism of the anterior mesentery artery containing live embryos.

B.—Four-year-old gelding with Strongylosis.

At autopsy December 17, 1918, there were found about 100 Gastrophilus with Strongylus and Cylicostomes.

C.—Four-year-old mare with Strongylosis.

At autopsy, there were found 14 *Ascaris equorum* in the duodenum and a moderately severe infestation of the cecum and colon with Strongylus and Cylicostomes.

E.—Three-year-old gelding with Strongylosis.

At autopsy, there was found a severe infestation of *Gastrophilus* spp. in the pylorus and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

F.—Four-year-old mare with Strongylosis.

At autopsy, October 21, 1918, there were found a few *Gastrophilus* spp. in the pylorus. There were 18 *Ascaris equorum* in the duodenum, 5 *Setaria equina* and 20 Strongylus in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

G.—Four-year-old mare with Strongylosis.

At autopsy, October 18, 1918, there were found a moderate number of *Gastrophilus* spp. in the pylorus, 2 Ascares in the duodenum, 1 Strongylus and 10 Setaria in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

H.—Three-year-old gelding with Strongylosis.

At autopsy, on October 24, 1918, there was found a moderate number of *Gastrophilus* spp. in the pylorus, one Strongylus and four Setaria in the abdominal cavity, and a very severe infestation of Strongylus and Cylicostomes in the cecum and colon.

D.—Three-year-old gelding in very poor condition, which, as far as could be ascertained, was due to the *Gastrophilus* spp.

At autopsy, there were found 1,030 *Gastrophilus* spp. in the pylorus, 12 Strongylus in the cecum and colon, and 3 Setaria in the abdominal cavity.

These cases are the ones that prompted this report. One of the main features of the positive diagnosis of internal parasitic diseases of man and the domestic animals apparently has been the presence of an eosinophilia.

On this basis, there being an absence of an eosinophilia and the animals having a high temperature, the cases were diagnosed as infectious equine anemia. The symptoms of the two diseases are very similar. Transmission experiments failed in a large number of cases, and, therefore, infectious equine anemia can be positively excluded. In a few months all the animals died or were killed. Seven of the animals had very severe infestations of Strongylus and Cylicostomes; the eighth harbored an enormous number of *Gastrophilus* spp.

At the time of the first examination the animals were not in bad physical condition. Some, in fact, were in good physical condition. Their resistance should have been good. From the facts presented by these cases it can be seen that other factors than the presence or absence of an eosinophilia are of great importance in the diagnosis of internal parasites of the horse.

TABLE III.—*Miscellaneous blood counts*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
12..	Feb. 2, 1919	90	7,642,000	11,420	39.2	49.2	7.4	4.2	0.0	0
	Mar. 8, 1919	90	7,964,000	7,039	34.2	60.2	2.4	3.2	0	0
	Mar. 27, 1919	90	6,836,000	7,200	32.0	60.0	7.4	.6	0	0
	Apr. 26, 1919	100	8,076,000	9,389	33.2	58.6	6.0	2.2	0	0
	May 16, 1919	60	5,864,000	7,349	39.2	55.6	6.4	.8	0	0
10..	Dec. 22, 1919	85	7,962,000	5,369	44.0	44.4	9.0	2.4	.2	0
	Jan. 3, 1919	80	6,934,000	5,387	10.6	82.4	6.0	1.0	0	0
	Average.....	85.0	7,325,400	6,693	33.2	58.6	6.3	2.0	.02	0

No. 12.—Twelve-year-old animal that apparently died from infectious equine anemia; positive diagnosis doubtful.

At autopsy, on May 24, 1919, there were found about 150 *Gastrophilus* spp., a moderate infestation of the cecum and colon with Strongylus and Cylicostomes, and a small aneurism of the anterior mesenteric artery containing a few live embryos.

No. 10.—Aged, healthy animal at time of first blood count; at the last the animal was very ill with septicemia.

At autopsy, on January 8, 1919, there were found 35 *Gastrophilus* spp., 2 Setaria in the abdominal cavity, and a few Strongylus and Cylicostomes in the cecum and colon.

TABLE IV.—*Blood counts of horses with acute cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
11..	Mar. 1, 1919	80	6,934,000	4,360	34.2	53.0	3.6	9.0	0.2	0
	Mar. 8, 1919	80	8,132,000	7,261	21.2	76.8	2.0	0	0	0
	Dec. 16, 1918	100	8,320,000	7,341	33.0	57.2	5.8	4.0	0	0
	Jan. 3, 1919	100	7,366,000	7,639	27.4	61.2	8.2	3.2	0	0
	Mar. 6, 1919	100	7,932,000	5,482	28.2	56.2	9.0	6.6	0	0
6...	Mar. 25, 1919	80	7,629,000	9,870	49.2	41.4	7.2	2.2	0	0
	Mar. 27, 1919	80	7,368,000	7,296	20.6	72.4	3.6	3.2	.2	0
	Apr. 8, 1919	80	6,884,000	7,329	22.4	73.6	3.6	.4	0	0
M..	Nov. 7, 1918	20	1,856,000	4,683	30.6	30.2	3.6	0	0	0
	Nov. 7, 1918	70	3,824,000	6,281	39.6	28.4	9.2	1.0	.4	31.4
N..	Nov. 16, 1918	50	4,864,000	9,669	29.0	36.7	3.3	1.0	0	30.0
L...	Nov. 7, 1918	80	4,320,000	6,993	22.6	66.2	9.2	2.0	0	0
	Oct. 8, 1918	100	8,634,000	6,981	26.6	63.0	4.6	3.8	0	0
	Nov. 2, 1918	90	7,824,000	9,873	33.6	61.0	3.2	2.2	0	0
758.	Nov. 14, 1918	60	6,408,000	6,321	14.2	82.2	3.6	0	0	0
	Nov. 15, 1918	70	6,488,000	7,369	23.6	71.4	4.2	.8	0	0
K..	June 2, 1919	50	4,872,000	6,329	35.4	60.2	4.2	.2	0	0
	June 17, 1919	30	3,486,000	5,317	28.4	57.4	11.2	3.0	0	0
J...	Mar. 26, 1919	80	.....	.....	37.8	55.4	2.6	4.2	0	0
	Average.....	73.6	6,285,600	7,021	29.3	58.1	5.4	2.5	.04	3.3

No. 11.—Aged animal with an acute experimental case of infectious equine anemia; inoculated February 19, 1919, and died March 23, 1919.

At autopsy, there were found about 100 *Gastrophilus* spp. in the pylorus, 1 Setaria in the abdominal cavity, and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 6.—Eight-year-old gelding with an acute experimental case of infectious equine anemia injected March 6, 1919.

At autopsy on March 10, 1919, there were found about 30 *Gastrophilus* spp. and 25 *Setaria equina*.

M.—Four-year-old mare with an acute case of infectious equine anemia.

At autopsy, on November 9, 1919, there were found a few *Gastrophilus* spp., one Setaria, a small worm-free aneurism of the anterior mesenteric

artery, and an enormous infestation of the cecum and colon with Strongylus and Cylicostomes.

N.—Ten-year-old mare with an acute case of infectious equine anemia.

At autopsy, on November 26, 1918, there were found a few *Gastrophilus* spp., eight *Ascaris equorum*, five Setaria, a few Strongylus and Cylicostomes in the cecum and colon, and a small anuerism of the anterior mesenteric artery containing live embryos.

L.—Aged mare with an acute case of infectious equine anemia.

At autopsy, December 24, 1918, there were found a few *Gastrophylus* spp. and a serious infestation of Strongylus and Cylicostomes in the cecum and colon.

No. 758.—Middle-aged gelding with an acute case of infectious equine anemia.

At autopsy, on November 17, 1918, there was found one Setaria in the abdominal cavity.

K.—Five-year-old gelding with an acute case of infectious equine anemia.

At autopsy, on June 17, 1919, there were found about 50 *Gastrophylus* spp., a small worm-free aneurism of the anterior mesenteric artery, and a severe infestation of the cecum and colon with Strongylus and Cylicostomes.

J.—Aged 7-year-old gelding with an acute case of infectious equine anemia.

At autopsy, on March 26, 1919, there were found 50 *Gastrophilus* spp., a few Strongylus and Cylicostomes in the cecum and colon, about 60 Setaria in the abdominal cavity, and an enormous aneurism of the anterior mesenteric artery containing large numbers of live embryos.

TABLE V.—*Blood counts of horses with subacute cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erythrocytes.	Leucocytes.	Lymphocytes.	Polymorphonuclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
1	Aug. 17, 1917...	45	1,940,000	1,888	48.9	46.6	3.7	0.2	0.6	0
21	June 9, 1919...	80	6,974,000	8,876	25.6	65.0	7.2	2.2	0	0
	July 3, 1919...	90	7,242,000	8,693	29.0	55.6	4.2	1.2	0	0
	Sept. 12, 1919...	80	6,464,000	7,560	37.6	52.2	9.0	1.2	0	0
	Average.	73.8	5,655,000	6,754	37.8	54.9	6.0	1.2	.15	0

No. 1.—At the time this blood examination was made the animal was in the early stage of a subacute case of infectious equine anemia.

At autopsy, August 21, 1917, there were found a few *Gastrophilus* spp. and a moderate infestation and Strongylus and Cylicostomes in the cecum and colon.

No. 21.—Aged mare with a subacute case of infectious equine anemia. At autopsy, on September 29, 1919, there were found severe infestations of Strongylus and Cylicostomes in the cecum and colon.

TABLE VI.—*Blood counts of horses with chronic cases of infectious equine anemia*

Tag No.	Date.	Hemoglobin.	Erithrocytes.	Leucocytes.	Lymphocytes.	Polymorphoclears.	Mononuclears.	Eosinophiles.	Mast cells.	Transitionals.
	Nov. 27, 1917	110	8,120,000	18,222	17.2	76.8	6.0	0	0	0
	Dec. 15, 1917	90	7,456,000	9,650	35.8	44.2	15.0	5.0	0	0
	May 2, 1918	89	7,728,000	8,200	30.4	61.6	7.6	1.2	.2	0
	May 16, 1918	75	7,422,000	10,200	32.6	60.2	5.0	2.2	0	0
	June 14, 1918	90	9,620,000	800	41.8	47.4	7.4	2.4	1.0	0
671	June 18, 1918	100	7,898,000	10,320	28.4	61.0	6.2	3.4	1.0	0
	June 24, 1918	78	9,080,000	5,310	36.2	55.6	3.8	4.0	.4	0
	Aug. 5, 1918	110	7,892,000	11,700	37.2	44.6	11.0	6.4	.8	0
	Aug. 26, 1918	87	8,072,000	7,633	37.0	48.6	7.4	6.2	.8	0
	Oct. 10, 1918	86	7,138,000	7,963	28.0	57.6	12.2	2.2	0	0
	Oct. 29, 1918	70	6,408,000	8,888	30.4	61.2	6.4	2.0	0	0
	Nov. 3, 1918	40	3,936,000	5,183	21.6	73.0	4.2	1.2	0	0
	Oct. 10, 1918	90	7,638,000	7,383	38.6	51.8	5.2	4.4	0	0
	Nov. 2, 1918	90	6,938,000	8,373	25.0	67.8	6.4	.8	0	0
	Jan. 2, 1919	80	4,864,000	7,639	33.4	57.2	8.0	1.4	0	0
	Jan. 11, 1919	80	5,836,000	7,939	33.0	52.2	9.2	5.6	0	0
	Jan. 28, 1919	40	5,120,000	7,631	33.6	58.0	7.0	1.4	0	0
	Mar. 13, 1919	90	7,264,000	7,360	16.0	77.2	6.4	.4	0	0
753	Mar. 19, 1919	80	5,872,000	8,390	54.4	42.6	3.0	0	0	0
	Mar. 25, 1919	70	5,984,000	6,390	53.6	42.6	1.2	2.6	0	0
	Apr. 26, 1919	80	6,984,000	5,387	37.4	58.2	3.6	.8	0	0
	May 16, 1919	90	7,638,000	5,931	34.2	51.2	9.4	5.2	0	0
	June 9, 1919	100	7,764,000	6,390	33.6	60.4	3.2	2.8	0	0
	July 3, 1919	100	7,634,000	7,961	38.6	57.2	4.4	.8	0	0
	Sept. 12, 1919	90	7,688,000	7,833	49.2	42.8	3.8	4.2	0	0
	May 2, 1918	74	8,032,000	7,300	41.6	50.2	4.8	2.8	.6	0
	May 11, 1918	85	6,848,000	7,480	28.6	61.4	4.4	5.6	0	0
	May 14, 1918	90	.....	.....	47.0	41.6	8.2	3.0	.2	0
	May 20, 1918	90	7,136,000	8,000	12.6	79.2	4.0	1.4	2.8	0
	May 23, 1918	90	7,840,000	7,333	29.4	67.2	4.8	.6	0	0
	May 28, 1918	95	7,232,000	8,000	43.6	35.0	16.8	2.2	2.2	0
672	June 3, 1918	90	7,326,000	7,960	33.0	58.2	4.4	4.2	.2	0
	June 12, 1918	77	5,184,000	6,120	41.6	45.4	8.4	3.6	1.0	0
	June 18, 1918	86	6,912,000	5,580	25.6	57.2	14.8	2.0	.4	0
	June 24, 1918	84	7,612,000	3,330	32.6	53.0	10.0	3.8	.6	0
	July 5, 1918	95	7,642,000	6,410	28.8	60.4	5.8	5.0	0	0
	July 8, 1918	68	6,080,000	7,200	28.2	63.4	4.4	3.0	1.0	0
	July 13, 1918	78	6,368,000	7,200	12.4	74.4	13.2	0	.4	0
24	Sept. 18, 1919	90	6,988,000	5,363	27.0	65.0	5.4	2.6	0	0
	Nov. 1, 1919	80	6,464,000	6,666	25.4	57.4	13.6	1.0	2.6	0
25	Dec. 24, 1917	80	7,264,000	6,733	30.2	49.4	13.6	6.8	0	0
	Mar. 18, 1920	100	8,328,000	4,328	No count made.					
	June 4, 1919	100	7,856,000	4,960	32.6	61.2	4.8	1.4	0	0
	July 3, 1919	80	6,398,000	7,333	36.6	53.8	8.2	1.4	0	0
18	Aug. 25, 1919	80	7,288,000	6,366	40.2	46.2	6.0	7.6	0	0
	Sept. 12, 1919	80	6,972,000	4,888	40.2	49.0	6.2	4.6	0	0
	Nov. 1, 1919	90	8,262,000	9,363	38.4	51.4	9.6	1.2	0	0
	Dec. 24, 1919	80	7,074,000	5,366	38.3	54.6	6.1	1.0	0	0
	Aug. 23, 1919	90	7,844,000	7,888	29.0	60.2	4.4	6.0	0	0
	Sept. 12, 1919	70	6,864,000	7,391	21.2	68.2	5.4	5.2	0	0
23	Oct. 9, 1919	60	5,834,000	7,200	42.2	47.2	6.4	4.2	0	0
	Nov. 1, 1919	80	6,824,000	4,266	39.4	44.4	13.0	3.2	0	0
	Dec. 24, 1919	70	6,876,000	4,866	40.4	46.2	11.0	2.4	0	0
	Average.....	84.0	7,119,000	7,225	33.5	56.0	7.3	2.9	.30	0

No. 671.—Aged gelding with a chronic case of infectious equine anemia.

At autopsy on November 4, 1918, there were found a few Strongylus and Cylicostomes in the cecum and colon and a small aneurism of the anterior mesenteric artery containing one live embryo.

No. 753.—Four-year-old colt with a chronic case of infectious equine anemia.

At autopsy there were found a moderate number of Strongylus and Cylicostomes in the cecum and colon and three *Setaria equina* in the abdominal cavity.

No. 672.—Middle-aged mare with a chronic case of infectious equine anemia.

At autopsy on July 14, 1918, there were found small numbers of *Gastrophilus* spp. and a moderate infestation of the cecum and colon with Strongylus and Cylicostomes.

No. 24.—Aged animal with a chronic case of infectious equine anemia.

At autopsy November 14, 1919, many *Gastrophilus* spp. were found, 10 Setaria, a serious infestation of Strongylus and Cylicostomes in the cecum and colon, and a small aneurism of the anterior mesenteric artery containing live embryos.

No. 25.—Twelve-year-old mare with a chronic case of infectious equine anemia.

At autopsy March 18, 1920, there were found a few *Gastrophilus* spp. and a moderate number of Strongylus and Cylicostomes in the cecum and colon.

No. 18.—Ten-year-old gelding with a chronic case of infectious equine anemia.

At autopsy on February 10, 1920, there were found three *Gastrophilus* spp., a few Strongylus and Cylicostomes, and a small worm-free anuerism of the anterior mesenteric artery.

No. 23.—Aged gelding with a chronic case of infectious equine anemia.

At autopsy on February 17, 1920, there were found about 150 *Gastrophilus* spp. and a moderate number of Strongylus and Cylicostomes in the cecum and colon.

Hadwen (7) has noted that the eosinophiles in cases of swamp fever (infectious equine anemia) are diminished in number and sometimes absent from the peripheral blood. In the cases here reported this is noticeable more especially in the acute cases than the chronic ones. There is very little, if any, decrease in the number of the eosinophiles in some of the more chronic cases.

In passing, it may well be said that in connection with some parasitic anaphylaxis experiments injection of ground up *Gastrophilus* spp. produced a marked local eosinophilia with severe abscess development. Local injection of ground up Strongylus produced a mild abscess

formation with practically no local eosinophilia. Mention of this is made in the hope that further observations will be made.

The work of Bücklers (*1*), Deglos (*3*), Giffin (*5*), Hadwen (*6*), Moore, Haring, and Cady (*8*), and Nazum (*9*) should also be consulted in connection with the study of eosinophilia.

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A SOURING OF BEEF CAUSED BY BACILLUS MEGATHERIUM

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The problem involved in the maintenance of a wholesome food supply for the public is without doubt one of paramount importance, concerning, as it does most intimately, the physical well-being of the Nation. Among the most difficult phases of this problem is that respecting the flesh foods. The long recognized fact of the communicability to man of certain animal diseases has made it imperative for the safeguarding of health that supervision be maintained over the extremely important industries engaged in the production of flesh foods.

But there are other considerations which stress the urgency of a proper watch over the character of the flesh foods to be offered to the people for consumption. Meats, to be acceptable to man, must be not only wholesome but palatable. It is quite conceivable that the flesh of a carcass which has satisfactorily passed a rigid inspection as to the existence of disease may subsequently be subjected to such a manner of handling as to render it utterly unsuitable for food. This is what actually occurs at times.

Aside from the objectionable qualities which may be normal or incidental to animals presented for slaughter, such as the sexual odors and flavors of certain adult male animals or odors and flavors produced by the use of ill-smelling feeds, etc., the flesh of animals from the time of slaughter to the time of consumption is continually susceptible to the acquisition of undesirable properties. Some of these may be acquired by absorption and others by the action of bacteria, as in ordinary putrefaction.

There is a condition known as sour beef, which is familiar alike to butchers and inspectors, and one which doubtless occasions no little economic loss, especially to small butchering establishments and retailers who may not be equipped with facilities for the storage of meat to prevent this alteration. In reference to this condition the following statement is quoted from Ostertag's "Handbook of Meat Inspection":[1]

> Stinking acid fermentation occurs in slaughtered domestic animals when the meat, while still warm, is stored in large pieces and in closed receptacles, or, in general, when it is subjected to conditions under which it can not cool. This alteration is characterized by the term "suffocated."

[1] OSTERTAG, Robert. HANDBOOK OF MEAT INSPECTION . . Translation by Earley Vernon Wilcox. ed. 2, rev., p. 746–747. New York. 1905.

It is not definitely known that the souring of beef referred to by Ostertag is identical with that which is discussed in this study.

Certainly the conservation of the interests of the consuming public in this regard can be accomplished only by precautions of a far-reaching nature, involving vigilance from the abattoir to the platter.

The common stock of knowledge as to the definite cause of the phenomenon known as sour beef is probably more or less vague.

A specimen of sour beef was sent to this laboratory from one of the large packing establishments with a view to determining definitely the cause of the condition in this specific instance, as the meat was very decidedly sour. The specimen was assigned the number 1510.

BACTERIOLOGICAL EXAMINATION OF SOUR BEEF

An effort was made to isolate any organisms that might be found in the interior of the specimen. This was accomplished in the following manner: A site was selected favorable to making a deep cut into the body of the specimen, and the surface was then seared for a radius of several inches with a hot platinum spatula. With a sterile scalpel and forceps this surface was removed to a depth of ½ inch. The newly exposed surface was then similarly seared, and with the aid of a fresh sterile scalpel and forceps a small cube of meat was aseptically taken out and placed into a tube of bouillon medium.

This bouillon culture showed appreciable growth at the end of 24 hours' incubation at 37° C. It was then shaken thoroughly, and a loopful of the suspension of organisms was distributed upon the surface of each of three plain agar plates.

Upon incubation for 24 hours at 37° C. these plates developed three types of organisms, which were later transplanted upon plain agar slants, and for convenience of identification designated as organism 1510–A, 1510–B, and 1510–C, respectively. Organism 1510–A proved to be *Staphylococcus albus*. Organism 1510–B was a medium short chain-forming and apparently spore-bearing bacillus. Organism 1510–C was a fungus.

An effort was then made to determine whether any one of these three organisms or any combinations thereof would artificially produce the sour-beef odor in meat as noticed in the original specimen. Sterile normal beef muscle pieces were placed in sterile plugged test tubes, and the following tests were applied.

Two tubes were inoculated with each organism and two tubes with each combination of two organisms. All of the tubes were incubated for 24 hours at 37° C., with the following results:

Organism 1510–A produced no odor.

Organism 1510–B produced a distinct sour-beef odor.

Organism 1510–C produced a musty, fungous odor.

Organisms 1510–A and B together produced a slight sour-beef odor.

Organisms 1510–A and C together produced a slight musty odor.

Organisms 1510–B and C together produced a blending of sour-beef and musty odors.

All of the meat pieces which were inoculated with the fungus were covered with a heavy overgrowth of this organism.

This experiment seemed to fix the responsibility for the souring of this specimen of beef upon organism 1510–B independently of the other two organisms. Both organisms 1510–A and 1510–C will therefore be

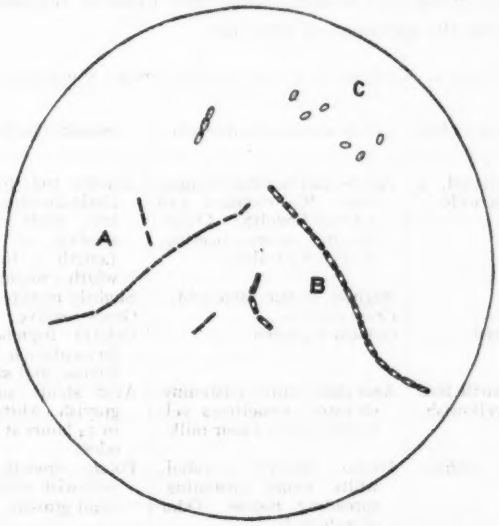

FIG. 1.—A, chains of the vegetative form of the organism; B, chains of organisms in beginning sporulation forms, containing immature spores; C, completely formed spores, in which stage the chain formation is invariably lost. Pen-and-ink drawing from microscope with aid of camera lucida.

disregarded from this point as incidental and of no significance in the souring of beef.

DESCRIPTION OF CAUSATIVE ORGANISM

Various diagnostic media were then employed to determine if possible the identity of organism 1510–B. It was found that the morphology and cultural characteristics of the organism corresponded more closely to those of *Bacillus megatherium* var. *Ravenellii* and *B. megatherium* var. *de Bary*, as described by Chester,[1] than to any other organism described.

The organism 1510–B may be briefly described as follows:

A round-ended, chain-forming, spore-bearing, Gram-positive, aerobic, slightly motile rod, from 2 to 5 microns long and 2 microns wide. During

[1] CHESTER, Frederick D. A MANUAL OF DETERMINATIVE BACTERIOLOGY. p. 271–272, 277. New York, London. 1909.

sporulation the organisms become symmetrically swollen, and the chains, naturally fragile, become even more so. The spores are equatorial. Figure 1 shows the microscopic appearance of organism 1510–B in the vegetative form (A) and in the immature (B) and mature (C) stages of sporulation. Isolated 24-hour colonies when slightly magnified are seen to be densely opaque at the center. The periphery is granular, with the the appearance of irregular circles composed of rhomboidal rugae.

In Table I there is given a comparative digest of the cultural characteristics of *Bacillus megatherium* var. *de Bary* and *B. megatherium* var. *Ravenellii*, as given by Chester,[1] along with those of organism 1510–B, recovered from the specimen of sour beef.

TABLE I.—*Comparison of cultural characteristics of organisms*

B. megatherium var. *de Bary.*	*B. megatherium* var. *Ravenellii.*	Organism 1510–B of sour beef.
Aerobe, flagellated, 2 to 5 microns wide.	Aerobe and facultative anaerobe. Rod-rounded, 3 to 5 times breadth. Chain-forming, spore-bearing. Rods not swollen.	Aerobic rod, rounded ends, chain-forming, spore-bearing. Rods symmetrically swollen at sporulation. Length 2 to 5 microns, width 2 microns.
	Slightly motile, ameboid...	Slightly motile.
Gram-positive.....	Gram-positive.....	Gram-positive.
Gelatin liquefied.....	Gelatin liquefied.....	Gelatin liquefied. No gas fermentation in dextrose, lactose, and saccharose.
Agar slant growth like *B. subtilis;* yellowish.	Agar slant, white, glistening, elevated, sometimes yellowish, odor of sour milk.	Agar slant, smooth, dull, grayish white, luxuriant in 24 hours at 37° C. Sour odor.
Potato, like *B. subtilis;* yellowish.	Potato, growth elevated, white, moist, glistening, spreading, rugose. Odor of stale milk.	Potato, smooth, glistening, yellowish white, moist, but scant growth.
	Litmus milk decolorized, slowly peptonized. Amphoteric. Alkaline.	Litmus milk coagulated, peptonized, decolorized. Neutral to P_H.
Indol negative, hydrogen sulphid (H_2S) positive.	Indol negative.....	Indol negative.
Habitat, cabbage infusion.	Habitat, soil.....	Habitat, sour beef.

Fermentation reactions as shown in Table II were obtained after 60 hours' incubation with *Bacillus megatherium* var. *de Bary* and organism 1510–B.

[1] CHESTER, Frederick D. OP. CIT.

TABLE II.—*Comparison of fermentation reactions*

Organism.	Dextrose.	Lactose.	Saccharose.	Mannite.	Xylose.	Levulose.
B. megatherium	Acid	Negative	Slight acid.	Negative	Negative	Acid.
1510–B	do	do	Acid	do	do	Do.

Organism.	Galactose.	Inulin.	Arabinose.	Dulcite.	Maltose.	Salacin.
B. megatherium	Acid	Negative	Negative	Negative	Acid	Acid.
1510–B	do	do	do	do	do	Negative.

CHEMICAL EXAMINATION OF SOUR BEEF

To determine the identity of the acid produced by organism 1510–B in the process of souring beef, the following preparations were made:

About 125 gm. of sterile raw meat pieces were inoculated with organism 1510–B and incubated for eight days. A corresponding container of sterile raw beef, uninoculated, was likewise treated as a control. At the end of eight days both specimens were removed from the incubator.

The inoculated specimen had acquired an intensely sour odor, characteristic of that previously produced by the organism in meat, while the control specimen was apparently unchanged.

As a control on the purity of the culture which had produced the souring, bouillon cultures were inoculated with fluids from the sour specimen. These cultures were plated, and eight representative colonies were "picked off" from the plates, each of which colonies subsequently developed, on agar slants, growths which invariably were true to type in morphology, cultural characteristics, and the ability to reproduce sour beef.

It was therefore concluded that this specimen was rendered sour by a pure culture of organism 1510–B.

This specimen and the uninoculated control specimen were then submitted to J. F. Couch, pharmacological chemist of the Pathological Division, Bureau of Animal Industry, for an identification of the acid produced in the meat by the organism 1510–B.

The report of his technic is as follows:

> Both samples were cooled in a refrigerator and were then extracted with cold ether. Upon evaporation of the ether extracts the residue from the normal beef deposited a small quantity of fatty matter which was neutral in reaction and had the odor of beef tallow. The residue obtained from the inoculated beef, however, was acid to litmus and carried the offensive putrefaction odor of the sour-beef specimen. From this small residue some evidence of propionic acid was obtained. The sour-beef specimen was, therefore, extracted with 300 milliliters of 2 per cent sulphuric-acid solution by digestion at a moderate temperature. The aqueous solution was filtered by suction, and the clear filtrate, which had the characteristic putrefaction odor, was

submitted to steam distillation. The distillate was acid and carried the putrefaction odor. This was titrated against *N*/5 sodium hydroxid, using phenolphthalein as indicator. Neutralization required 50.78 milliliters. The solution of the sodium salt was then concentrated by distilling off the solvent. The distillate carried the putrefaction odor and was neutral to litmus. The residue was transferred to a glass evaporating dish, dried to constant weight, at 105° C. and weighed. Weight of sodium salt, 0.9704 gm. Calculated for $C_2H_5CO_2Na$, 0.9855 gm. The identity of the acid was confirmed by conversion into the barium and silver salts, by solubility and organoleptic tests, and by Dyer's color test.[1]

PATHOGENICITY AND TOXIN PRODUCTION

Tests were conducted to determine the pathogenicity of the organism recovered from sour beef and to ascertain whether it produces toxin in meat or on artificial media.

Two guinea pigs were inoculated with a normal saline suspension of a culture of the organism taken from a plain agar slant, one guinea pig receiving the inoculation subcutaneously and the other intraperitoneally. No ill effects were produced by this organism.

A third guinea pig was fed pieces of meat thoroughly soured with the organism and was also "drenched" with a heavy saline suspension of the organisms. This animal lived.

Two guinea pigs were inoculated intraperitoneally each with 1 cc. of a heavy saline suspension of the washed organisms from a 24-hour agar culture. They were not affected.

Two guinea pigs were inoculated intraperitoneally with the supernatant fluid of a 24-hour bouillon culture of the organism but showed no ill effects.

These tests indicate that this organism is not pathogenic for guinea pigs and does not produce any appreciable toxin in ordinary media or raw beef under laboratory conditions.

COMPARATIVE ODOR PRODUCTION OF OTHER ORGANISMS

An experiment was conducted to compare the odor produced on meat by this organism with any odors that might be produced by other organisms selected at random. Sterile normal meat pieces were inoculated with various organisms in order to determine whether this characteristic sour-beef odor would be reproduced or approximated by any of them on raw meat. These inoculated meat pieces were incubated for four days at 37° C. The following organisms failed to produce any perceptible odor:

Bacillus pyocyaneus.
Bacillus of Priesz and Nocard.
Staphylococcus albus.
Staphylococcus citreus.

[1] DYER, D. C. A NEW METHOD OF STEAM DISTILLATION FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS, INCLUDING A SERIES OF COLORIMETRIC QUALITATIVE REACTIONS FOR THEIR IDENTIFICATION. In Jour. Biol. Chem., v. 28, no. 2, p. 469. 1917.

B. bronchisepticus.
B. typhi murium.
Actinomyces bovis.
Moeller's grass bacillus.
B. lactimorbis.
B. pullorum.
B. mesentericus.
B. prodigiosus.
B. enteritidis.
B. coli communis.
B. coli communior.
B. icteroides.
Streptococcus mastitis.
B. gallinarium.
B. subtilis.

The following organisms produced odors as described:

Saccharomyces, slight sweetish odor.
Blastomyces, distinct yeasty odor.
Bacillus vulgatus, chestnut odor.

Organism 1510–B again produced the typical sour-beef odor.

The odor produced by organism 1510–B is strikingly characteristic; it is readily distinguishable from the yeasty or putrefactive odors, and it is not produced on raw beef by any of the other organisms used in this experiment.

THERMAL AND OXYGEN REQUIREMENTS

A facultative test of the beef-souring property of organism 1510–B with regard to temperature and oxygen tension was next applied. Meat pieces were inoculated with the organism and treated as follows for four days, with the results noted in each case:

(1) Aerobically at room temperature. Produced perceptible sour-beef odor, though not so strong as when incubated.

(2) Aerobically in refrigerator (0° to 4.5° C.). Produced a faint suggestion of sour-beef odor.

(3) Anaerobically in incubator. Produced no odor.

(4) Under partially reduced oxygen tension in incubator. No odor.

It was concluded from these results that the organism 1510–B is an aerobic saprophyte with a wide range of vegetative temperatures but with an optimum of about 37° C.

SEROLOGY

It was then decided to determine if possible the existence of a specific or group agglutination reaction which would establish a relationship between the organism isolated from sour beef and *Bacillus megatherium*.

Cultures of *Bacillus megatherium* No. 270 and 734 were obtained from the American Museum of Natural History of New York City. No. 734 was the de Bary strain, but the identity of No. 270 was unknown.

Two rabbits were hyperimmunized against organism 1510–B by intravenous injections of 0.5 cc. of a washed saline suspension of the organism on the first day, 1 cc. on the fifth day, 2 cc. on the twelfth day, and 2 cc. on the nineteenth day. Samples of blood serum were then taken and were tested against carbolized normal salt solution suspensions of the organisms (agglutinating fluids). A sample of normal rabbit serum was also tested against each organism as a control. After 24 hours' incubation at 37° C, the tests were read, as shown in Table III.

TABLE III.—*Agglutination tests*

Animal.	*B. megatherium*, 270.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	?	?
Hyperimmune rabbit B	+	+	+	+	+	+
Normal rabbit C	?	?	−	−	−	−

Animal.	*B. megatherium*, var. *de Bary*, 734.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	?	−
Hyperimmune rabbit B	+	+	+	+	−	−
Normal rabbit C	−	−	−	−	−	−

Animal.	Organism 1510-B.					
	0.04	0.02	0.01	0.005	0.002	0.001
Hyperimmune rabbit A	+	+	+	+	+	+
Hyperimmune rabbit B	+	+	+	+	+	+
Normal rabbit C	−	−	−	−	−	−

Anaerobic cultures of the three organisms on 3 per cent glycerin agar were incubated 18 hours at 37° C. with no apparent growth. At 60 hours' incubation these cultures all show minute dewdrop colonies. The same cultures were then incubated aerobically for 24 hours at 37°, producing a luxuriant growth, typical of *Bacillus megatherium*.

The apparent identity of the organism 1510–B as *Bacillus megatherium* now appeared to be clearly established by its morphological and cultural likeness to the known organism of that name. It remained to be seen, however, whether the phenomenon of the souring of beef could be reproduced by a known culture of *B. megatherium* derived from a

source other than sour beef. For a determination of this problem both cultures obtained from the American Museum of Natural History were used for the inoculation of sterile specimens of raw beef. A third specimen was inoculated with organism 1510–B. After 24 hours' incubation it was found that the typical odor of sour beef had become pronounced in each of the samples inoculated. It was therefore concluded that *B. megatherium* is capable of producing the condition commonly known as sour beef.

MICROSCOPIC APPEARANCE OF SOUR BEEF

Several small pieces of artificially soured beef were prepared for sectioning by the paraffin method. Sections were made, mounted, and

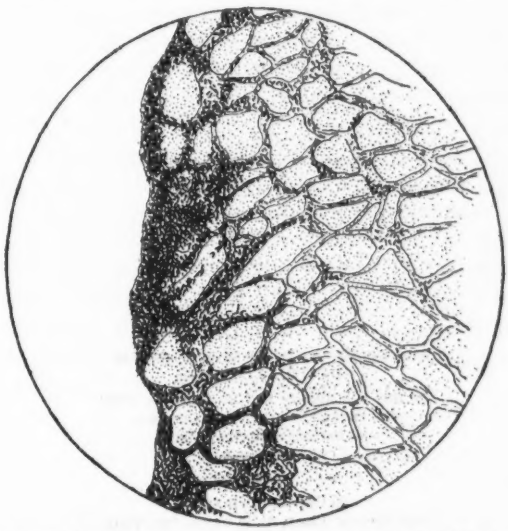

FIG. 2.—A cross section of beef muscle inoculated with organism 1510–B, stained by the Gram-Weigert method to show bacteria. Pen-and-ink drawing from microscope with aid of camera lucida.

stained by the Gram-Weigert method to show bacteria. Figure 2 is a reproduction of a pen-and-ink drawing made with camera lucida from one of the sections as seen under the microscope. The organisms are seen in large numbers on the exterior surface of the section, a fact which may be accounted for by the aerobic nature of the organisms. As they penetrated into the interior of the meat, growing between the muscle fibers, their multiplication was greatly inhibited by the anaerobic condition of the interior of the tissue, and yet a few persevering organisms may be seen to have penetrated well into the tissue.

CONCLUSIONS

The phenomenon known as the souring of beef is a bacterial one.

The organism responsible for the souring of beef is *Bacillus megatherium.*

Bacillus megatherium will sour beef under a wide range of temperatures, but not in the absence of oxygen.

In the souring of beef by *Bacillus megatherium* propionic acid is produced.

Bacillus megatherium is nonpathogenic for experimental animals (rabbits and guinea pigs) and does not produce an appreciable amount of toxin when propagated upon raw beef.

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